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(54) Title: NITRATE ESTERS AND THEIR USE FOR NEUROLOGICAL CONDITIONS

(57) Abstract

Aliphatic nitrate esters having a sulfur or phosphorus atom β or γ to a nitrate group and their congeners having efficacy as neuroprotective agents are described. Preferred nitrate esters may be synthesized by nitration of a 3-bromo-1,2-propanediol, and subsequent reaction to yield the desired mono-, di- or tetra-nitrate ester.

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NITRATE ESTERS AND THEIR USE FOR NEUROLOGICAL CONDITIONS

CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation in part of our earlier filed application, Serial No. 08/658,145 filed 4 June 1996, the disclosure of which is incorporated herein by reference.

FIELD OF INVENTION

This invention relates to novel aliphatic nitrate esters and use thereof as neuroprotective agents. More particularly this invention relates to nitrate esters bearing a sulfur or phosphorus atom β or γ to a nitrate group and their congeners which have therapeutic utility as neuroprotective agents and/or cognition enhancers.

BACKGROUND OF INVENTION

The nitrate ester, glyceryl trinitrate (GTN) or nitroglycerin, has been used as a vasodilator in the treatment of angina pectoris for over a hundred years, and the dominant contemporary belief is that GTN exerts its therapeutic effect through *in vivo* release of nitric oxide (NO). Other organic nitrates, such as isosorbide dinitrate, have also been identified as effective and clinically important vasodilators. NO itself has been identified as Endothelium Derived Relaxing Factor (EDRF) and several classes of compounds, for example nitrosothiols, in addition to organic nitrates, have been proposed as NO donors or NO prodrugs. Well-known examples of these classes of compounds and GTN itself have been suggested to demonstrate neurotoxic or neuroprotective effects by dint of interactions with the redox modulatory site of the *N*-methyl-D-aspartate (NMDA) excitatory amino acid receptor. Thus GTN is firstly a potent vasodilator and secondly possesses neuroprotective properties. Several attempts have been made to increase the efficacy or potency of alternative nitrate esters as vasodilators relative to GTN, for example, by incorporation of propanolamine or cysteine functionalities. However, no attempt has been made to separately regulate the vasodilatory and neuroprotective effects of GTN. Indeed, postural hypertension, weakness and other signs of cerebral ischemia are adverse effects, associated with the vasodilatory effects of GTN and observed in treatment, which are highly contraindicative of GTN itself as a clinically useful neuroprotective therapeutic agent.

In as much as the potent vasodilatory effects of nitrate esters may prove (a) deleterious to or alternatively (b) synergistic with the neuroprotective effects of GTN, it is postulated herein that regulation of these two effects is required for development of new and useful neuroprotective therapeutic agents. Further, it is postulated that such regulation may be achieved through use of nitrate esters incorporating sulfur-containing or phosphorus-

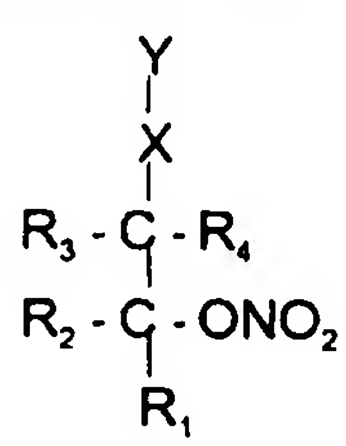
containing functionalities into the structure of the nitrate esters or through use of their congeners. Interaction of nitrate esters with amino acid neurotransmitter receptors, including the NMDA receptor, will provide examples of compounds with neuroprotective properties, but modulation of the γ-aminobutyric acid (GABA) receptor response will provide examples of nitrate esters capable of cognition enhancement. These postulates are based, in part, on bioassay data on such compounds. Thus, there is a need for synthetic aliphatic nitrate esters containing sulfur or phosphorus functionalities or their congeners as new and useful therapeutic agents for use in neuroprotection and/or cognition enhancement. It will be appreciated, therefore, that these compounds can be used for treatment of: stroke; Parkinson's disease; Alzheimer's disease; Huntington's disease; multiple sclerosis; amyotrophic lateral sclerosis; AIDS-induced dementia; epilepsy; alcoholism; alcohol withdrawal; drug-induced seizures; viral/bacterial/fever-induced seizures; trauma to the head; hypoglycemia; hypoxia; myocardial infarction; cerebral vascular occlusion; cerebral vascular hemorrhage; hemorrhage; environmental excitoxins of plant, animal and marine origin; dementias of all type, trauma, drug-induced brain damage, aging.

OBJECT OF INVENTION

It is an object of the present invention to provide novel aliphatic nitrate esters bearing a sulfur or phosphorus moiety β or γ to a nitrate group, or congeners thereof. Another object of the present invention is to provide methods for making the novel S- or P-containing nitrate esters and their novel congener nitrate esters. Yet another object of the present invention is to provide novel drugs for use as neuroprotective agents and/or cognition enhancers.

BRIEF DESCRIPTION OF INVENTION

By one aspect of this invention there is provided aliphatic nitrate esters containing at least one nitrate group, in which a S or P atom is situated β or γ to a nitrate group, or congeners thereof, having the general formula:



where X is CH_2, O, NH, NMe, CN, NHOH, N_2H_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, N_3, S, SCN, $SCN_2H_2(R_5)_2$, $SCN_2H_3(R_5)$, $SC(O)N(R_5)_2$, $SC(O)NHR_5$, SO_3M, SH, SR_7, SO_2M, $S(O)R_8$,

$S(O)_2R_9$, $S(O)OR_8$, $S(O)_2OR_9$, PO_3M_2, $P(O)(OR_5)(OR_6)$, $P(O)(OR_6)(OM)$, $P(O)(R_5)(OR_8)$, $P(O)(OM)R_5$, CO_2M, CO_2H, CO_2R_{11}, $C(O)$, $C(O)R_{12}$, $C(O)(OR_{13})$, PO_2M, $P(O)(OR_{14})$, $P(O)(R_{13})$, SO, SO_2, $C(O)(SR_{13})$, SR_4, or SSR_4;

Y is SCN, $SCN_2H_2(R_5)_2$, $SC(O)NHR_5$, $SC(O)N(R_5)_2$, SR_4, SR_{10}, SSR_{10}, SO_2M, SO_3M, PO_3HM, PO_3M_2, $P(O)(OR_5)(OR_6)$, or $P(O)(OR_6)(OM)$, CN, N_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, CO_2M, CO_2H, CO_2R_{11}, $C(O)R_{12}$, $C(O)(SR_{13})$, or does not exist;

R_5, R_6, R_8, R_9, R_{10}, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, are the same or different alkyls containing 1-12 carbon atoms which may contain 1-4 ONO_2 substituents or C_1 or C_2 connections to R_1 - R_3 in cyclic derivatives;

R_7, R_{11} are C_1 - C_8, alkyl or acyl;

R_2 and R_4 are the same or different and selected from H, ONO_2, C_1-C_4 alkyl optionally bearing 1-3 nitrate groups, and acyl groups ($-C(O)R_{10}$);

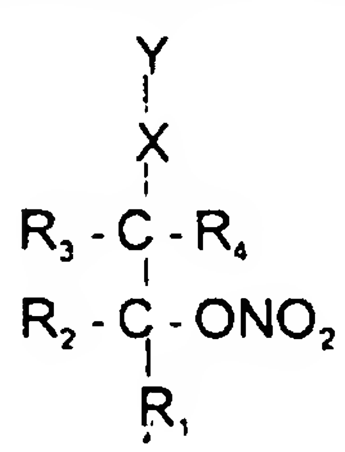
R_1 and R_3 are the same or different and selected from H, C_1-C_4 alkyl and chains, which may include one O, linking R_1 and R_3 to form pentosyl, hexosyl, cyclopentyl or cycohexyl rings, which rings optionally bear hydroxyl substituents; and

M is H, Na^+, K^+, NH_4^+ or $N^+H_nR_{11(4-n)}$ where n is 0-3;

and with the proviso that, when X is O, Y is not COR_{12}, and with the proviso that, when R_3 is H, R_6 is not ethyl or n-butyl;

and pharmaceutically acceptable salts thereof.

By another aspect of this invention there is provided a pharmaceutical composition comprising an effective amount of an aliphatic nitrate ester having the formula:



where X is CH_2, O, NH, NMe, CN, NHOH, N_2H_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, N_3, S, SCN, $SCN_2H_2(R_5)_2$, $SCN_2H_3(R_5)$, $SC(O)N(R_5)_2$, $SC(O)NHR_5$, SO_3M, SH, SR_7, SO_2M, $S(O)R_8$, $S(O)_2R_9$, $S(O)OR_8$, $S(O)_2OR_9$, PO_3M_2, $P(O)(OR_5)(OR_6)$, $P(O)(OR_6)(OM)$, $P(O)(R_5)(OR_8)$, $P(O)(QM)R_5$, CO_2M, CO_2H, CO_2R_{11}, $C(O)$, $C(O)R_{12}$, $C(O)(OR_{13})$, PO_2M, $P(O)(OR_{14})$, $P(O)(R_{13})$, SO, SO_2, $C(O)(SR_{13})$, SR_4, or SSR_4;

Y is SCN, $SCN_2H_2(R_5)_2$, $SC(O)NHR_5$, $SC(O)N(R_5)_2$, SR_4, SR_{10}, SSR_{10}, SO_2M, SO_3M, PO_3HM, PO_3M_2, $P(O)(OR_5)(OR_6)$, or $P(O)(OR_6)(OM)$, CN, N_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, CO_2M, CO_2H, CO_2R_{11}, $C(O)R_{12}$, $C(O)(SR_{13})$, or does not exist;

R_5, R_6, R_8, R_9, R_{10}, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, are the same or different alkyls containing

1-12 carbon atoms which may contain 1-4 ONO_2 substituents or C_1 or C_2 connections to R_1 - R_3 in cyclic derivatives;

R_7, R_{11} are C_1 - C_8, alkyl or acyl;

R_2 and R_4 are the same or different and selected from H, ONO_2, C_1-C_4 alkyl optionally bearing 1-3 nitrate groups, and acyl groups (-C(O)R_{10});

R_1 and R_3 are the same or different and selected from H, C_1-C_4 alkyl and chains, which may include one O, linking R_1 and R_3 to form pentosyl, hexosyl, cyclopentyl or cycohexyl rings, which rings optionally bear hydroxyl substituents; and

M is H, Na^+, K^+, NH_4^+ or $N^+H_nR_{11(4-n)}$ where n is 0-3;

and with the proviso that, when X is O, Y is not COR_{12}, and with the proviso that, when R_3 is H, R_6 is not ethyl or n-butyl;

and the pharmaceutically acceptable salts thereof, in admixture with a physiologically acceptable carrier therefor.

By yet another aspect of this invention there is provided a method for effecting neuroprotection in a patient in need thereof comprising administering to said patient an effective amount of an aliphatic nitrate ester having the formula:

$$\begin{array}{c} Y \\ | \\ X \\ | \\ R_3 - C - R_4 \\ | \\ R_2 - C - ONO_2 \\ | \\ R_1 \end{array}$$

where X is CH_2, O, NH, NMe, CN, NHOH, N_2H_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, N_3, S, SCN, $SCN_2H_2(R_5)_2$, $SCN_2H_3(R_5)$, SC(O)N$(R_5)_2$, SC(O)NHR_5, SO_3M, SH, SR_7, SO_2M, S(O)R_8, S$(O)_2R_9$, S(O)OR_8, S$(O)_2$OR_9, PO_3M_2, P(O)(OR_5)(OR_6), P(O)(OR_6)(OM), P(O)(R_5)(OR_8), P(O)(OM)R_5, CO_2M, CO_2H, CO_2R_{11}, C(O), C(O)R_{12}, C(O)(OR_{13}), PO_2M, P(O)(OR_{14}), P(O)(R_{13}), SO, SO_2, C(O)(SR_{13}), SR_4, or SSR_4;

Y is SCN, $SCN_2H_2(R_5)_2$, SC(O)NHR_5, SC(O)N$(R_5)_2$, SR_4, SR_{10}, SSR_{10}, SO_2M, SO_3M, PO_3HM, PO_3M_2, P(O)(OR_5)(OR_6), or P(O)(OR_6)(OM), CN, N_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, CO_2M, CO_2H, CO_2R_{11}, C(O)R_{12}, C(O)(SR_{13}), or does not exist;

R_5, R_6, R_8, R_9, R_{10}, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, are the same or different alkyls containing 1-12 carbon atoms which may contain 1-4 ONO_2 substituents or C_1 or C_2 connections to R_1 - R_3 in cyclic derivatives;

R_7, R_{11} are C_1 - C_8, alkyl or acyl;

R_2 and R_4 are the same or different and selected from H, ONO_2, C_1-C_4 alkyl optionally bearing 1-3 nitrate groups, and acyl groups (-C(O)R_{10});

R_1 and R_3 are the same or different and selected from H, C_1-C_4 alkyl and chains, which may include one O, linking R_1 and R_3 to form pentosyl, hexosyl, cyclopentyl or cycohexyl rings, which rings optionally bear hydroxyl substituents; and

M is H, Na^+, K^+, NH_4^+ or $N^+H_nR_{11(4-n)}$ where n is 0-3;

and with the proviso that, when X is O, Y is not COR_{12}, and with the proviso that, when R_3 is H, R_6 is not ethyl or n-butyl;

and pharmaceutically acceptable salts thereof.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 is a graph showing the effect of GTN with added L-cysteine (2mM) on soluble guanylyl cyclase (Gcase) activity in the 105,000g supernatant fraction of rat aorta homogenate. Experimental incubations were performed at 37°C for 10 min. Bars represent the mean ± standard errors calculated separately for each point.

Figure 2 is a graph showing the effect of compound 1; neat (triangles); with added L-cysteine (2mM, diamonds); with added dithiothreitol (2mM, DTT, squares); on soluble Gcase activity in the 105,000g supernatant fraction of rat aorta homogenate, normalized to maximal GTN response carried out in identical Gcase preparations. Experimental incubations were performed at 37°C for 10 min. Bars represent the mean ± standard errors calculated separately for each point.

Figure 3 is a graph showing the effect of compound 2; neat (diamonds); with added L-cysteine (2mM, triangles); with added dithiothreitol (2mM, DTT, squares); on soluble Gcase activity in the 105,000g supernatant fraction of rat aorta homogenate, normalized to maximal GTN response carried out in identical Gcase preparations. Experimental incubations were performed at 37°C for 10 min. Bars represent the mean ± standard errors calculated separately for each point.

Figure 4 is a graph showing the effect of compound 3; neat (diamonds); with added L-cysteine (2mM, triangles); with added dithiothreitol (2mM, DTT, squares); on soluble Gcase activity in the 105,000g supernatant fraction of rat aorta homogenate, normalized to maximal GTN response carried out in identical Gcase preparations. Experimental incubations were performed at 37°C for 10 min. Bars represent the mean ± standard errors calculated separately for each point.

Figure 5 is a graph showing the effect of compound 7; neat (diamonds); with added L-cysteine (2mM, triangles); with added dithiothreitol (2mM, DTT, squares); on soluble Gcase activity in the 105,000g supernatant fraction of rat aorta homogenate, normalized to maximal GTN response carried out in identical Gcase preparations. Experimental incubations were performed at 37°C for 10 min. Bars represent the mean ± standard errors

calculated separately for each point.

Figure 6 is a graph showing the effect of compound 4; neat (diamonds); with added L-cysteine (2mM, triangles; 5mM, circles); with added dithiothreitol (2mM, DTT, squares); on soluble Gcase activity in the 105,000g supernatant fraction of rat aorta homogenate, normalized to maximal GTN response carried out in identical Gcase preparations. Experimental incubations were performed at 37°C for 10 min. Bars represent the mean ± standard errors calculated separately for each point.

Figure 7 is a graph showing the effect of compound 8; neat (diamonds); with added L-cysteine (2mM, triangles); with added dithiothreitol (2mM, DTT, squares); on soluble Gcase activity in the 105,000g supernatant fraction of rat aorta homogenate, normalized to maximal GTN response carried out in identical Gcase preparations. Experimental incubations were performed at 37°C for 10 min. Bars represent the mean ± standard errors calculated separately for each point.

Figure 8 is a graph showing the effect of compound 10; neat (diamonds); with added L-cysteine (2mM, triangles); with added dithiothreitol (2mM, DTT, squares); on soluble Gcase activity in the 105,000g supernatant fraction of rat aorta homogenate, normalized to maximal GTN response carried out in identical Gcase preparations. Experimental incubations were performed at 37°C for 10 min. Bars represent the mean ± standard errors calculated separately for each point.

Figure 9 is a graph showing the effect of compound 11; neat (diamonds); with added L-cysteine (2mM, triangles); with added dithiothreitol (2mM, DTT, squares); on soluble Gcase activity in the 105,000g supernatant fraction of rat aorta homogenate, normalized to maximal GTN response carried out in identical Gcase preparations. Experimental incubations were performed at 37°C for 10 min. Bars represent the mean ± standard errors calculated separately for each point.

Figure 10 is a graph showing the the relaxation induced by compound 2 in untreated and GTN tolerant rat aorta.

Figure 11 is a graph showing the relaxation induced by compounds 3 (a) and 4 (b) in isolated rat aorta.

Figure 12 is a graph showing the comparison of concentration response curves for GTN and compound 5 in isolated rat aorta. Data points represent mean ± SD (n=5-6).

Figure 13 is a graph showing the relaxation induced by *tert*-butyl nitrosothiol in isolated rat aorta.

Figure 14 is a graph showing the relaxation induced by compound 5 in untreated and GTN-tolerant isolated rat aorta.

Figure 15 is a graph showing the $GABA_A$ receptor-activated membrane current

recorded in an oocyte expressing the α1β2γ2L receptor isoform. GABA (10 μM) was applied until the peak steady-state current response was obtained. Compound 2 (Bunte salt, 10-100 μM) was pre-applied for 30 seconds prior to exposure of the oocyte to GABA. At 100 μM the Bunte salt produced a 55% inhibition of the response to 10 μM GABA. Similar results were obtained in 5 different oocytes.

Figure 16 is a graph showing that nitric oxide donors have no effect on $GABA_A$ receptors expressed in *Xenopus* oocytes. Diethylamine nonoate salt (DEA) and *t*-butylnitrosothiol (t-BuSNO) which both spontaneously release nitric oxide in aqueous solution, had no effect on $GABA_A$ receptor-activated membrane current in an oocyte expressing the α1β2γ2L receptor isoform. In contrast, nitroglycerin (GTN) produced a reversible inhibition of the GABA response.

Figure 17 is a graph showing that the concentration response relationship for activation of the $GABA_A$ receptor is altered in a non-competitive manner by organic nitrate esters. The Bunte salt 2 (pre-applied for 30 seconds) decreased the peak current amplitude from 302 nA to 150 nA. However, the EC_{50} concentration (GABA concentration producing 50% of the maximal response) for GABA was not changed. This suggests that organic nitrates produce an allosteric alteration in $GABA_A$ receptor activation.

Figure 18 is a graph showing the effect of GTN (0.2 or 0.4 mg/hr) implanted subcutaneously one hour prior to an infusion of NMDA into the substantia nigra on striatal tyrosine hydroxylase (TH) activity.

Figure 19 is a graph showing the effect of GTN (0.4 mg/hr) implanted subcutaneously one hour after an infusion of NMDA into the substantia nigra on striatal (TH) activity.

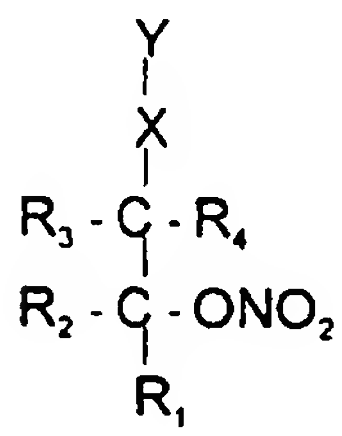
Figure 20 is a graph showing the percent decrease in striatal TH activity. Male Sprague-Dawley rats were stereotactically infused with NMDA into the substantia nigra and the striata were dissected and assayed for TH activity. The striata of each animal were compared to express neurotoxicity as a percent decrease in TH activity of the ipsilateral striatum as compared to the contralateral striatum. Animals pretreated with GTN showed significant amounts of neuroprotection; whereas, animals pretreated with losartan did not show any evidence of neuroprotection.

Figures 21 (a) and (b) are graphs showing the blood pressure profiles. Male Sprague-Dawley rats with aortic catheters were connected to pressure transducers which recorded blood pressure for 4 to 8 hours. Mean arterial pressure (MAP) curves for typical animals are represented above. (a) The animals treated with transdermal 0.4 mg/hour GTN patches implanted in the dorsal neck region, showed a 15% decrease in MAP 250 minutes post-implantation. (b) Animals treated with a single 30 mg/kg intraperitoneal injection of

losartan showed a 20% decrease in MAP 250 minutes after injection. From these data, treatment protocols for the NMDA infusion experiments were generated.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Simple nitrate esters according to this invention contain one or more nitrate groups in which a S or P atom is situated β or γ to a nitrate group or their congeners. Accordingly, a general formula for the esters of this invention is:



where X is CH_2, O, NH, NMe, CN, NHOH, N_2H_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, N_3, S, SCN, $SCN_2H_2(R_5)_2$, $SCN_2H_3(R_5)$, $SC(O)N(R_5)_2$, $SC(O)NHR_5$, SO_3M, SH, SR_7, SO_2M, $S(O)R_8$, $S(O)_2R_9$, $S(O)OR_8$, $S(O)_2OR_9$, PO_3M_2, $P(O)(OR_5)(OR_6)$, $P(O)(OR_6)(OM)$, $P(O)(R_5)(OR_8)$, $P(O)(OM)R_5$, CO_2M, CO_2H, CO_2R_{11}, C(O), $C(O)R_{12}$, $C(O)(OR_{13})$, PO_2M, $P(O)(OR_{14})$, $P(O)(R_{13})$, SO, SO_2, $C(O)(SR_{13})$, SR_4, or SSR_4;

Y is SCN, $SCN_2H_2(R_5)_2$, $SC(O)NHR_5$, $SC(O)N(R_5)_2$, SR_4, SR_{10}, SSR_{10}, SO_2M, SO_3M, PO_3HM, PO_3M_2, $P(O)(OR_5)(OR_6)$, or $P(O)(OR_6)(OM)$, CN, N_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, CO_2M, CO_2H, CO_2R_{11}, $C(O)R_{12}$, $C(O)(SR_{13})$, or does not exist;

R_5, R_6, R_8, R_9, R_{10}, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, are the same or different alkyls containing 1-12 carbon atoms which may contain 1-4 ONO_2 substituents or C_1 or C_2 connections to R_1 - R_3 in cyclic derivatives;

R_7, R_{11} are C_1 - C_8, alkyl or acyl;

R_2 and R_4 are the same or different and selected from H, ONO_2, C_1-C_4 alkyl optionally bearing 1-3 nitrate groups, and acyl groups ($-C(O)R_{10}$);

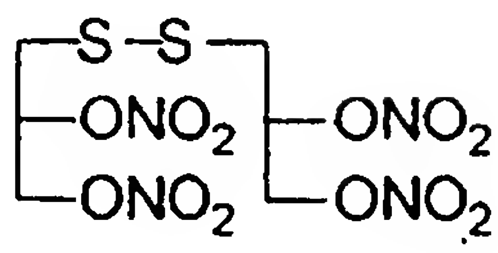
R_1 and R_3 are the same or different and selected from H, C_1-C_4 alkyl and chains, which may include one O, linking R_1 and R_3 to form pentosyl, hexosyl, cyclopentyl or cycohexyl rings, which rings optionally bear hydroxyl substituents; and

M is H, Na^+, K^+, NH_4^+ or $N^+H_nR_{11(4-n)}$ where n is 0-3;

and with the proviso that, when X is O, Y is not COR_{12}, and with the proviso that, when R_3 is H, R_6 is not ethyl or n-butyl;

and the pharmaceutically acceptable salts thereof.

Compounds according to the present invention fall into nine main categories having the formulae: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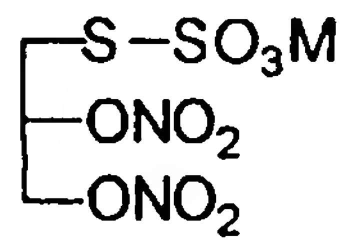
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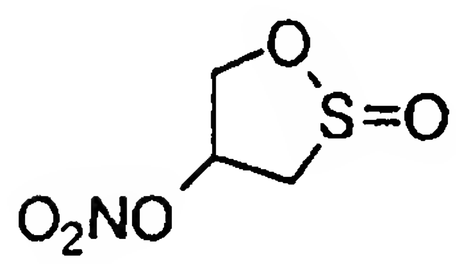
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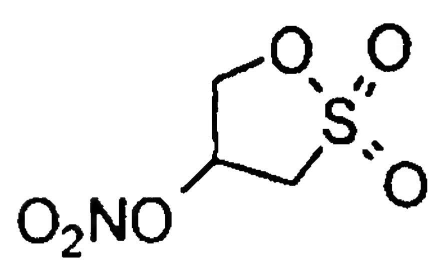
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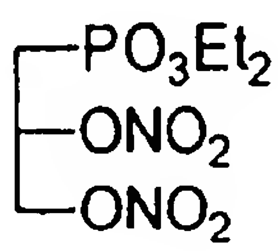
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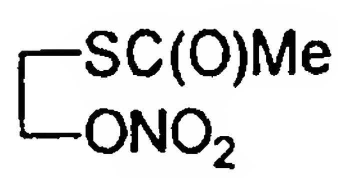
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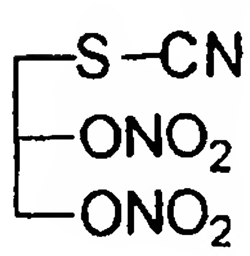
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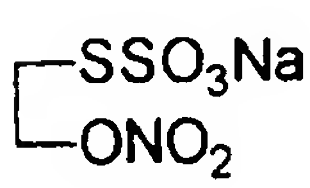
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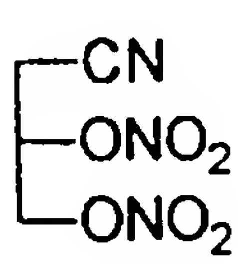
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The compound of formula 1 was synthesized via the Bunte salt (2). The synthesis of compound 2 proceeded from a 3-halopropane-1,2-diol by dropwise addition into a cold mixture of nitric acid (68-70%, 4.0eq) and sulfuric acid (95%, 4.0 eq) in methylene chloride (50 mL) and reaction at room temperature for 30 minutes. The organic layer was separated,

washed, dried and concentrated to yield a yellow oil which was purified by silica gel flash chromatography to give a 45% yield of 3-halopropane-1,2-diol dinitrate: either 3-bromopropane-1,2-diol dinitrate (compound 10) or 3-chloropropane-1,2-diol dinitrate (compound 11). The Bunte salt (compound 2) was prepared by reacting either nitrate 10 or 11 with an equimolar portion of $Na_2S_2O_3$ in 3:1 MeOH/H_2O at 50°C for 10 hours and subsequently purifying by silica gel flash chromatography. The Bunte salt 2 was oxidized with a small molar excess of hydrogen peroxide H_2O_2 (30%) in an ethanol: water mixture (1:1) with a catalytic amount of sulfuric acid for 2 days. Extraction with methylene chloride, concentration, and purification by silica gel flash chromatography yielded the tetranitrate (1): The ^{13}C NMR spectra of compounds 1 and 2 revealed 6 and 3 signals respectively, as expected from the presence of two chiral centres in compound 1 and only one in compound 2 (Table 1). The Bunte salt mononitrate 8 was synthesized by an analogous method to the Bunte salt dinitrate 2, in this case, starting from 2-chloroethyl nitrate or 2-bromoethyl nitrate.

Nitration of bis-(2,3-dihydroxypropyl) disulfide in the biphasic CH_2Cl_{12}/aq. HNO_3/ H_2SO_4, medium described above, yielded, after chromatographic silica gel purification, 10% and 5% yields of the sultine 3 and sultone 4, respectively. NMR spectra obtained for these products were highly solvent-dependent and were similar to those of the glycerol dinitrates, but with the significant difference that the large geminal coupling is associated with the upfield rather than the downfield methylene protons (Table 1). Definitive structure identification rested upon mass spectral data: soft chemical ionization with Cl^- ion capture determined compounds 3 and 4 to be the sultine and sultone, respectively.

The compound of formula 5 was synthesized by two methods. In the first method, a 3-halopropane-1, 2-diol was silylated to provide a substrate for the Arbuzov reaction with triethyl phosphite. The resulting phosphonate was nitrated using nitronium tetrafluoroborate to yield the product 5, which was isolated after silica flash chromatography. In the second method, an Arbuzov reaction with 1-halo-2,3-epoxypropane yielded the 1-phosphono-2,3-epoxypropane, which was converted to the dinitrate 5 using the same nitrating procedure detailed for synthesis of compound 1. Isolation after chromatography yielded the product 5 with: ^{31}P NMR δ=23ppm; ^{13}C NMR δ=16, 27, 62, 71, 75ppm; ^{1}H NMR δ= 1.3, 2.2, 4.1, 4.4-5.0, 5.5ppm.

The compound of formula 6 was synthesized from bromoethyl nitrate by stirring with an excess of a thioacetate salt in the presence or absence of a crown ether catalyst in a methanolic solution at room temperature overnight. The product was isolated in 50% yield after silica gel flash chromatography.

The compound of formula 7 was synthesized from the dinitrate 10, by stirring of dinitrate 10 and a thiocyanate alkali metal salt (1 equivalent) in acetone at room temperature

over a number of days. The white precipitate produced was removed by filtration and the filtrate concentrated and passed through a silica gel flash chromatography column, yielding the desired product as a colourless oil (50%) (Table 1).

The compound of formula 9 was synthesized from the dinitrate 10, by stirring of dinitrate 10 and a cyanide alkali metal salt in acetone at room temperature over a number of days, with or without the aid of phase transfer catalyst or crown ether. The precipitate was removed from the reaction mixture by filtration and the filtrate concentrated and passed through a silica gel flash chromatography column, yielding the desired product as a colourless oil: ^{1}H NMR ($CDCl_3$) 5.30-5.43(1H, m), 4.82-4.90 (1H, dd), 4.65-4.75 (1H, dd), 3.85-3.97 (2H, dd).

Table 1 NMR, IR, m.p. and mass spec. characteristics of nitrates[a]

m.p.	^{1}H-NMR(ppm)[b]	^{13}C-NMR(ppm)[b]	IR(cm^{-1})	MS(m/z, intensity%)
1 liquid	5.43-5.55(2H, m), 4.84-4.93(2H, m), 4.60-4.69(2H, dd, J=6, 13Hz), 2.97-3.16(4H, m).	77.08/77.00, 69.33/69.29, 37.05/36.89.	(neat),1634, 1270, 1042, 995, 855.	(Cl, Cl^-), 429(M+Cl, 100), 393(M-1, 10).
2 86°C(dec.)	(DMSO-d_6), 5.75-5.80(1H, m), 4.99-5.07(1H, dd, J=3, 13Hz), 4.77-4.86(1H, dd, J=6, 13 Hz), 3.20-3.23(2H, d, J=7 Hz)	(DMSO-d_6), 79.02, 70.97, 32.04.	(KBr), 1638, 1449, 1378, 1351, 1290, 1210, 1042, 654.	(ES^+, Na), 323(M+Na, 53).
3 64-65°C	5.84-5.90(1H, m), 4.98-5.06(1H, dd, J=4, 12Hz), 4.77-4.83(1H, d, J=12Hz), 3.50-3.58(1H, dd, J=2, 15Hz), 3.31-3.42(1H, dd, J=7, 15Hz).	80.49, 75.07, 64.04.	(KBr), 1649, 1339, 1287, 1122, 926.	(Cl, Cl^-), 202(M+Cl, 100)
4 65-66°C	5.80-5.87(1H, m), 4.67-4.75(1H, dd, J=5, 11 Hz), 4.50-4.57(1H, dd, J=2, 11 Hz), 3.69-3.80(1H, dd, J=8, 15 Hz), 3.35-3.44(1H, dd, J=3, 15 Hz).	76.95, 69.88, 48.66.	(KBr), 1651, 1344, 1286, 1139, 937.	(Cl, Cl^-), 218(M+Cl, 100).
6 liquid	4.52-4.56 (2H, t, J=6.5Hz), 3.16-3.20 (2H, t, J=6.5Hz), 2.37 (1H, s)	70.8, 30.4, 26.0	-	(Cl), 218(M+H, 23), 119(M-NO_2, 100).
7 liquid	5.45-5.55(1H, m), 4.87-4.95(1H, dd), 4.65-4.74(1H, dd), 3.18-3.38(2H, m)	110.19, 76.40, 68.76, 32.09	-	(Cl, Cl^-), 258(M+Cl, 100).
8 164-165°C	4.77 (2H, t, J=6Hz), 3.35 (2H, t, J=6Hz)	-	-	(Cl), 218(M+H, 23), 119(M-NO_2, 100).
10 liquid	5.41-5.43(1H, m), 4.84-4.88(1H, dd, J=3, 13Hz), 4.68-4.73(1H, dd, J=6, 13Hz), 3.55-3.57 (2H, dd, J=0.5, 6Hz)	76.95, 69.16, 26.19	(neat) 1647, 1428, 1288, 1271, 1010, 835	-

a. All compounds were characterized by elemental analysis and/or high resolution mass spec., HPLC and NMR analysis for homogeneity.
b. $CDCl_3$ was used as solvent for ^{1}H-, ^{13}C-NMR, unless otherwise indicated.

Activation of soluble guanylyl cyclase (Gcase) by nitrates 1-5, 7 was assayed, employing partially purified enzyme freshly prepared from rat aorta homogenates, using the radioimmunoassay method described by Bennett *et al.*, *Can. J. Physiol. Pharmacol.* (1992) 70, 1297, the disclosure of which is incorporated herein by reference. Dose-response curves were obtained for Gcase activation by nitrates 1-5, 7 and GTN in the presence and absence of cysteine and dithiothreitol (DTT; both 2mM). The data from these curves are summarized in Figures 1-9, which give: concentrations of nitrates required to give a response equivalent to the maximal response seen for GTN+cysteine; the maximal response measured for each nitrate; and, where applicable, potency. The Gcase assay data show that dinitrate 2 activates Gcase, with a submillimolar EC-50 in the absence of any added thiol, in contrast to GTN which requires added cysteine (Figs 1, 3). Compounds 2 and 4 also activate Gcase in the presence of DTT in contrast to GTN (Figs 3, 6). Activation of Gcase by compound 5 was cysteine-dependent and the response was approximately one third of that observed with GTN. Activation of Gcase by compound 7 was cysteine-dependent and the response was very low (EC-50>1mM) (Fig. 7). The activity of the tetranitrate 1 was again low and entirely equivalent to glycerol-1,2-dinitrate in this assay (Fig. 2). Relative to GTN itself, a wide range of potency is observed for the novel organic nitrate esters. No activation of Gcase by glycerol mononitrates is observed in this assay at the concentrations of nitrate employed.

In order to extend the Gcase data, the relaxing effects of nitrates 2,3, 4 and 5 on rat aortic tissue were examined. Thoracic aortic strips were prepared from male Sprague-Dawley rats (Charles-River, Canada) as described in McGuire *et al.*, *J. Exp. Ther.* (1994) 271, 708 and Stewart *et al.*, *Can. J. Physiol. Pharmacol.* (1989) 67, 403; incorporated herein by reference. Tissues were contracted submaximally with phenylephrine (0.1μM) and exposed to various concentrations of nitrovasodilator to obtain concentration response curves. In this intact tissue assay, nitrates 2, 3, 4 and 5 were observed to cause relaxation of the tissue with a maximal relaxant response equal to that obtained with GTN. However, the compounds differed in potency with EC-50 values of 3.9μM (Fig.10), 3.4μM (Fig.11), 9.1μM (Fig.11) and 0.2μM (Fig.12) for compounds 2, 3, 4 and 5 respectively. The EC-50 values for a nitrosothiol (*tert*-butyl nitrosothiol, Fig.13) and for GTN itself (Fig.12) were 11.2μM and 14nM, respectively. Compounds 2 and 5 were tested for their ability to cause vascular relaxation in tissues that had been made tolerant to the relaxant effect of GTN. GTN tolerance was induced by incubating tissues with high concentrations of GTN (0.5mM GTN for 30 min). Under these conditions, the maximal relaxant effect of compounds 2 (Fig.10) and 5 (Fig.14) was not significantly different

from that of untreated tissue. The EC-50 for relaxation was increased approximately threefold, but the difference was not statistically significant.

The direct effects of organic nitrates on amino acid neurotransmitter receptors has been tested using the *Xenopus* oocyte expression system and two-electrode voltage-clamp recording methods. Human recombinant γ-aminobutyric acid type A ($GABA_A$) receptors composed of α1β2γ2L subunits were expressed in *Xenopus* oocytes as described in Reynolds and Maitra, *Eur. J. Pharmacol.* (1996) 314, 151-156, incorporated herein by reference. $GABA_A$ receptor-activated membrane current was recorded in individual oocytes, and modulation of this current by GTN and nitrate esters described in this application was assessed. GTN and the Bunte salt, compound 2, were found to inhibit $GABA_A$ receptor-activated membrane current with similar potencies. Concentrations of 10-100 μM of the nitrates produced a concentration-dependent inhibition of the GABA response (Fig.15). This effect appears to be unrelated to the production or release of nitric oxide, as nitric oxide generating compounds do not mimic this effect of GTN or the Bunte salt 2 to inhibit $GABA_A$ receptor function (Fig. 16). Organic nitrates such as GTN and the Bunte salt 2 do not compete with GABA for binding to the $GABA_A$ receptor. Rather, they produce an allosteric modulation of the receptor that decreases the maximal current without changing the apparent affinity of the receptor for GABA (Fig. 17). Other organic nitrates described in this application have been found to have similar inhibitory effects on $GABA_A$ receptor-activated membrane current. In behavioural models of learning and memory, drugs which decrease $GABA_A$ receptor function improve performance on learning and memory tasks (Venault, P.G., Chapouthier, L., Prado de Carvalho and Rossier, J., *Encephale*, (1992) 18, 655). Thus, the behavioural effect of organic nitrates, developed to act as modulators of $GABA_A$ receptor function, will be to improve memory performance and cognition in patient populations. It will be appreciated, therefore, that these nitrate esters can be used for treatment of: stroke; dementias of all type; trauma; drug-induced brain damage; and aging.

GTN, owing to its structural properties, is capable of preventing damage or loss of neurons induced by an excitotoxic insult involving excessive activation of glutamate receptors on brain neurons. The neuroprotective action of GTN on the nigrostriatal dopamine neurons of the rat was examined in a model of dopaminergic neuron excitotoxicity using a previously described method: Connop *et al.*, *Brain Research* (1995) 676, 124-132, incorporated herein by reference. An infusion of 15 nmoles of the glutamate receptor agonist N-Methyl-D-Aspartate (NMDA) into the right substantia nigra of adult rats (300-325 g) was made. A subdermal patch containing GTN (to release GTN at the rate of 0.2 or 0.4 mg/hr) was implanted under the skin

in the dorsal neck either before or one hour after the focal infusion of NMDA (Fig.19). In separate experiments, losartan, a vasodilator drug that acts by blocking angiotensin II type I receptors, replaced the administration of GTN prior to NMDA infusion. The blood pressure of treated animals was monitored. Four days after the NMDA injection, activity of tyrosine hydroxylase (TH), a marker for dopamine neurons, was measured in the striatum on injected and uninjected sides of the brain. In the absence of GTN, injection of NMDA produced 53% decrease in ipsilateral striatal TH activity, indicating a significant depletion of dopaminergic neurons projecting to the striatum (Fig.18). Treatment with NMDA coupled to 0.2 and 0.4 mg/hr GTN resulted in 25% and 18% decrease in striatal TH activity, respectively (Fig.18). In animals treated with GTN (O.4 mg/hr) 1 hour after the NMDA infusion, the decrease in striatal TH was 15% (Fig.19). Comparison of these values with those obtained in the absence of GTN treatment revealed a significantly lower depletion of dopaminergic neurons following treatment with GTN. The data showed that GTN significantly reduces NMDA-induced excitotoxic damage to dopaminergic neurons. In the losartan-treated (30 mg/kg) animal the decrease in TH activity was 53%. This value was not different from that obtained in the NMDA-injected animals. The data indicated that losartan does not inhibit NMDA-induced dopaminergic neuron damage. In the blood pressure experiments, GTN (0.4mg/hr) treatment resulted in a 15% decrease in the mean arterial pressure (MAP) and losartan (30 mg/kg) treatment resulted in a 20% decrease in the MAP (Fig.20). The results of blood pressure and toxicity experiments show that the ability of GTN to decrease NMDA-induced dopaminergic neuron damage is not related to decreases in blood pressure, since both GTN and losartan decreased blood pressure but only GTN produced neuroprotection. It will be appreciated, therefore, that these nitrate esters can be used for treatment of: stroke; Parkinson's disease; Alzheimer's disease; Huntington's disease; multiple sclerosis; amyotrophic lateral sclerosis; AIDS-induced dementia; epilepsy; alcoholism; alcohol withdrawal; drug-induced seizures; viral/bacterial/fever-induced seizures; trauma to the head; hypoglycemia; hypoxia; myocardial infarction; cerebral vascular occlusion; cerebral vascular hemorrhage; hemorrhage; environmental excitoxins of plant, animal and marine origin.

It will be appreciated by those skilled in the art, that any nitrate ester in which vasodilatory potency is reduced and neuroprotective potency increased, represents a new and useful therapeutic agent for use in neuroprotection, including in treatment of stroke; Parkinson's disease; Alzheimer's disease; Huntington's disease; multiple sclerosis; amyotrophic lateral sclerosis; AIDS-induced dementia; epilepsy; alcoholism; alcohol withdrawal; drug-induced seizures; viral/bacterial/fever-induced seizures; trauma to the head; hypoglycemia; hypoxia;

myocardial infarction; cerebral vascular occlusion; cerebral vascular hemorrhage; hemorrhage; environmental excitoxins of plant, animal and marine origin. GTN itself, proposed as a neuroprotective agent, has no clinical utility as a neuroprotective agent in therapy owing to its extraordinarily high vasodilatory potency.

It will be appreciated, additionally, by those skilled in the art, that the use in therapy of any nitrate ester in cognition enhancment, represents a new and useful therapeutic treatment for cognition enhancement, including in treatment of: stroke; dementias of all type, trauma, drug-induced brain damage, and aging.

A composition of the invention for therapeutic use comprises a nitrate ester and a physiologically acceptable carrier therefor. The term "physiologically acceptable carrier" as used herein includes diluents, solvents or dispersion media such as, for example, water, saline, glycerol and the like, as well other carriers, such as, for example, liposomes. Liposomes include water-in-oil-in-water emulsions as well as conventional liposomes (Strejan *et al.* (1984) *J. Neuroimmunol* 7, 27). A composition of the invention may be co-administered to a subject in need thereof with agent(s) to prevent its inactivation by enzymes, acids or other natural conditions. Such agents include coatings, enzyme inhibitors and the like. A composition of the invention may include agent(s) to facilitate delivery of the active compound, or which delay absorption, such as, for example, gelatin. A composition of the invention may also include antibacterial or antifungal agents, such as, for example, thimersol, parabens or the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional medium or agent is incompatible with the active compound, its use in the therapeutic compositions of the invention is contemplated.

Methods of the invention provide administration of an active compound (nitrate ester or salt thereof) to a subject in need thereof in a biologically compatible form. That is, the active compound is administered in a form in which any toxic effects are outweighed by the therapeutic effects of the active compound. Administration of a therapeutically active amount of a composition of the present invention means an amount effective, at dosages and for periods of time necessary, to achieve the desired result. A therapeutically active amount of nitrate esters of the invention may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the nitrate esters to elicit a desired response in the individual.

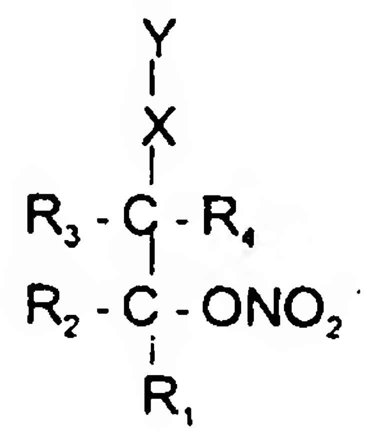
The active compound (nitrate ester or salt thereof) may be administered in any convenient manner, such as, for example, by injection (subcutaneous, intravenous,

intraventricular, intrathecally, intramuscular, intracavernous, etc.), oral administration, inhalation, transdermal application, lingual, sublingual, buccal, nasal, or rectal administration. The active compound may also be administered parenterally or intraperitoneally. In certain preferred embodiments of the invention, transdermal patches (containing from about 0.05 mg to about 1 g per single dosage form) and ointments or creams are suitable for transdermal and topical administration.

Although this invention is described in detail with reference to preferred embodiments thereof, these embodiments are offered to illustrate but not to limit the invention. It is possible to make other embodiments that employ the principles of the invention and that fall within its spirit and scope as defined by the claims appended hereto.

We Claim:

1. Aliphatic nitrate esters and therapeutically acceptable salts derived therefrom, containing at least one nitrate group, in which a S or P atom is situated β or γ to a nitrate group, or their congeners, having a general formula:



where X is CH_2, O, NH, NMe, CN, NHOH, N_2H_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, N_3, S, SCN, $SCN_2H_2(R_5)_2$, $SCN_2H_3(R_5)$, $SC(O)N(R_5)_2$, $SC(O)NHR_5$, SO_3M, SH, SR_7, SO_2M, $S(O)R_8$, $S(O)_2R_9$, $S(O)OR_8$, $S(O)_2OR_9$, PO_3M_2, $P(O)(OR_5)(OR_6)$, $P(O)(OR_6)(OM)$, $P(O)(R_5)(OR_8)$, $P(O)(OM)R_5$, CO_2M, CO_2H, CO_2R_{11}, C(O), $C(O)R_{12}$, $C(O)(OR_{13})$, PO_2M, $P(O)(OR_{14})$, $P(O)(R_{13})$, SO, SO_2, $C(O)(SR_{13})$, SR_4, or SSR_4;

Y is SCN, $SCN_2H_2(R_5)_2$, $SC(O)NHR_5$, $SC(O)N(R_5)_2$, SR_4, SR_{10}, SSR_{10}, SO_2M, SO_3M, PO_3HM, PO_3M_2, $P(O)(OR_5)(OR_6)$, or $P(O)(OR_6)(OM)$, CN, N_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, CO_2M, CO_2H, CO_2R_{11}, $C(O)R_{12}$, $C(O)(SR_{13})$, or does not exist;

R_5, R_6, R_8, R_9, R_{10}, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, are the same or different alkyls containing 1-12 carbon atoms which may contain 1-4 ONO_2 substituents or C_1 or C_2 connections to R_1 - R_3 in cyclic derivatives;

R_7, R_{11} are C_1 - C_8, alkyl or acyl;

R_2 and R_4 are the same or different and selected from H, ONO_2, C_1-C_4 alkyl optionally bearing 1-3 nitrate groups, and acyl groups ($-C(O)R_{10}$);

R_1 and R_3 are the same or different and selected from H, C_1-C_4 alkyl and chains, which may include one O, linking R_1 and R_3 to form pentosyl, hexosyl, cyclopentyl or cycohexyl rings, which rings optionally bear hydroxyl substituents; and

M is H, Na^+, K^+, NH_4^+ or $N^+H_nR_{11(4-n)}$ where n is 0-3;

and with the proviso that, when X is O, Y is not COR_{12}, and with the proviso that, when R_3 is H, R_6 is not ethyl or n-butyl.

2. Aliphatic nitrate esters as claimed in claim 1, wherein:

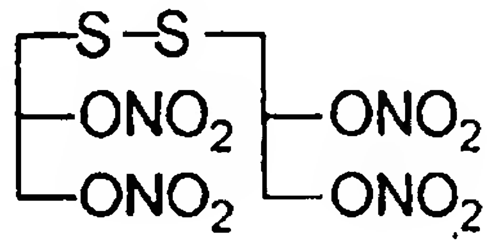
X is selected from the group consisting CH_2, O, NH, NMe, S, SCN, $SC(O)N(R_5)_2$,

$SC(O)NHR_5$, SO_3M, PO_2M, PO_3M_2, SH, SR_7, $P(O)(OR_5)(OR_6)$, $P(O)(OR_6)(OM)$, $P(O)(R_5)(OR_8)$, $P(O)(OM)R_5$, SO_2M, $S(O)R_8$, $S(O)_2R_9$, $S(O)OR_8$ or $S(O)_2OR_9$; and

Y is selected from the group consisting of SCN, $SC(O)N(R_5)_2$, $SC(O)NHR_5$, SR_4, SR_{10}, SSR_{10}, SO_2M, SO_3M, PO_3HM, PO_3M_2, $P(O)(OR_5)(OR_6)$, or $P(O)(OR_6)(OM)$, or does not exist.

3. Aliphatic nitrate esters as claimed in claim 1, wherein Y is not present.

4. Aliphatic nitrate esters as claimed in claim 1, having a formula selected from the group consisting of: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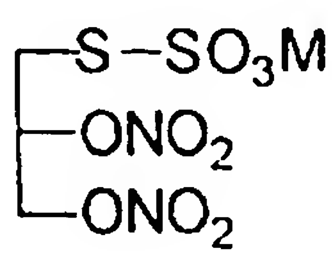
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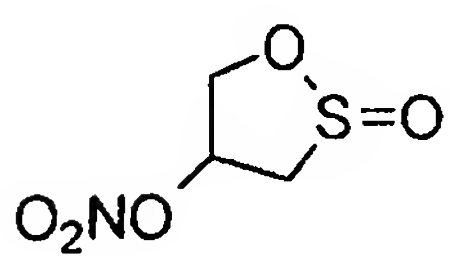
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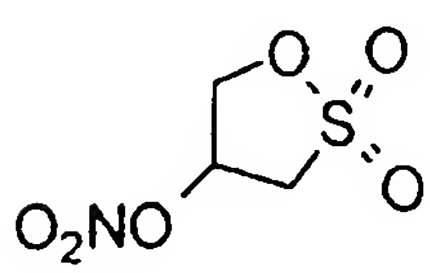
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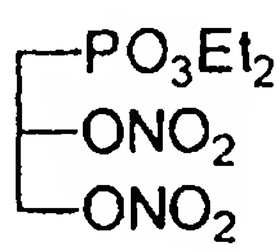
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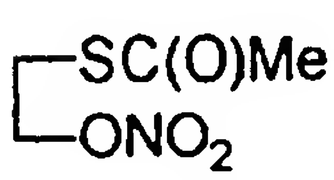
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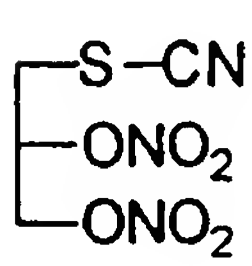
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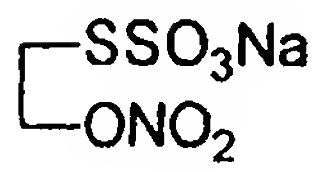

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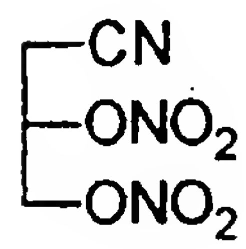
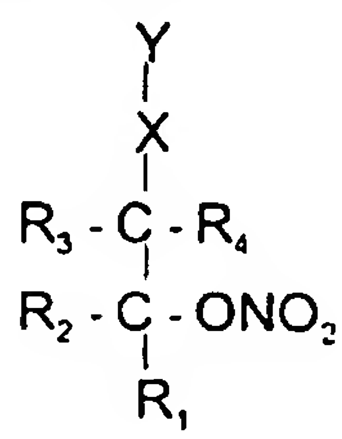


(9)

5. A pharmaceutical composition comprising an effective amount of an aliphatic nitrate ester or a pharmaceutically acceptable salt thereof, having the formula:



where X is CH_2, O, NH, NMe, CN, NHOH, N_2H_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, N_3, S, SCN, $SCN_2H_2(R_5)_2$, $SCN_2H_3(R_5)$, $SC(O)N(R_5)_2$, $SC(O)NHR_5$, SO_3M, SH, SR_7, SO_2M, $S(O)R_8$, $S(O)_2R_9$, $S(O)OR_8$, $S(O)_2OR_9$, PO_3M_2, $P(O)(OR_5)(OR_6)$, $P(O)(OR_6)(OM)$, $P(O)(R_5)(OR_8)$, $P(O)(OM)R_5$, CO_2M, CO_2H, CO_2R_{11}, C(O), $C(O)R_{12}$, $C(O)(OR_{13})$, PO_2M, $P(O)(OR_{14})$, $P(O)(R_{13})$, SO, SO_2, $C(O)(SR_{13})$, SR_4, or SSR_4;

Y is SCN, $SCN_2H_2(R_5)_2$, $SC(O)NHR_5$, $SC(O)N(R_5)_2$, SR_4, SR_{10}, SSR_{10}, SO_2M, SO_3M, PO_3HM, PO_3M_2, $P(O)(OR_5)(OR_6)$, or $P(O)(OR_6)(OM)$, CN, N_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, CO_2M, CO_2H, CO_2R_{11}, $C(O)R_{12}$, $C(O)(SR_{13})$, or does not exist;

R_5, R_6, R_8, R_9, R_{10}, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, are the same or different alkyls containing 1-12 carbon atoms which may contain 1-4 ONO_2 substituents or C_1 or C_2 connections to R_1 - R_3 in cyclic derivatives;

R_7, R_{11} are C_1 - C_8, alkyl or acyl;

R_2 and R_4 are the same or different and selected from H, ONO_2, C_1-C_4 alkyl optionally bearing 1-3 nitrate groups, and acyl groups ($-C(O)R_{10}$);

R_1 and R_3 are the same or different and selected from H, C_1-C_4 alkyl and chains, which may include one O, linking R_1 and R_3 to form pentosyl, hexosyl, cyclopentyl or cycohexyl rings, which rings optionally bear hydroxyl substituents; and

M is H, Na^+, K^+, NH_4^+ or $N^+H_nR_{11(4-n)}$ where n is 0-3;

and with the proviso that, when X is O, Y is not COR_{12}, and with the proviso that, when R_3 is H, R_6 is not ethyl or n-butyl;

in admixture with a pharmaceutically acceptable carrier therefor.

6. A pharmaceutical composition as claimed in claim 5, herein

X is selected from the group consisting CH_2, O, NH, NMe, S, SCN, $SC(O)N(R_5)_2$, $SC(O)NHR_5$, SO_3M, PO_2M, PO_3M_2, SH, SR_7, $P(O)(OR_5)(OR_6)$, $P(O)(OR_6)(OM)$, $P(O)(R_5)(OR_8)$, $P(O)(OM)R_5$, SO_2M, $S(O)R_8$, $S(O)_2R_9$, $S(O)OR_8$ or $S(O)_2OR_9$; and

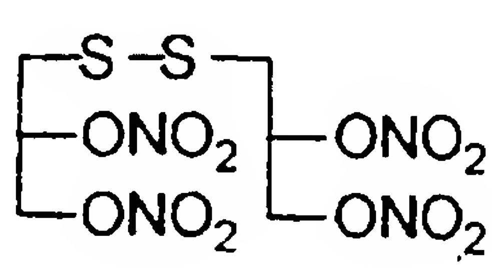
Y is selected from the group consisting of SCN, $SC(O)N(R_5)_2$, $SC(O)NHR_5$, SR_4, SR_{10}, SSR_{10}, SO_2M, SO_3M, PO_3HM, PO_3M_2, $P(O)(OR_5)(OR_6)$, or $P(O)(OR_6)(OM)$, or does not exist.

7. A pharmaceutical composition as claimed in claim 5, wherein

X is selected from a group consisting of CH_2, O, CN, NH, NMe, N_2H_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, N_3, CO_2M, CO_2H, CO_2R_{11}, C(O), $C(O)R_{12}$, $C(O)(OR_{13})$, S, SO, PO_2M, $P(O)(OR_{14})$, $P(O)(R_{13})$, SO_2, $S(O)R_8$, $S(O)_2R_9$, or SO_3M; and

Y is selected from the group consisting of CN, N_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, CO_2M, CO_2H, CO_2R_{11}, $C(O)R_{12}$, $C(O)(SR_{13})$, SR_4, SR_{10}, SSR_{10}, SO_2M, SO_3M, PO_3HM, PO_3M_2, $P(O)(OR_6)(OM)$, or $P(O)(OR_5)OR_6$, or does not exist.

8. A pharmaceutical composition as claimed in claim 5, wherein said nitrate ester has a formula selected from the group consisting of:



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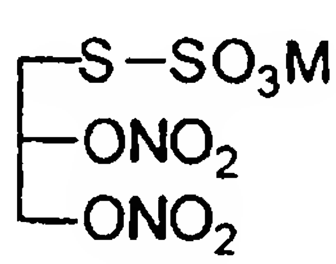
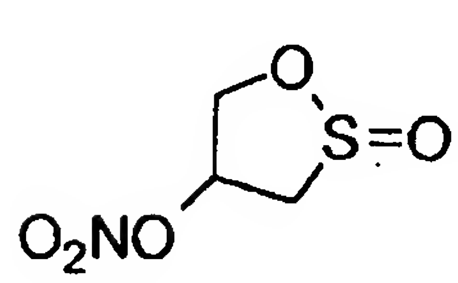
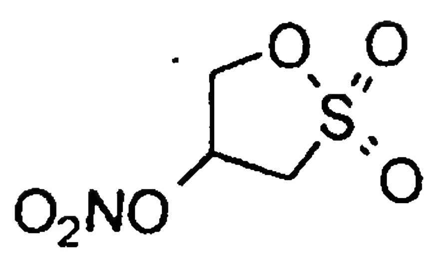


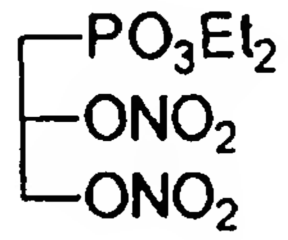
(2)



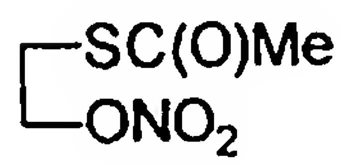
(3)



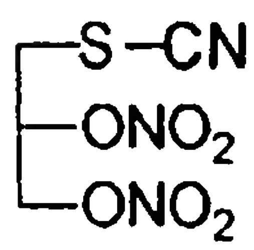
(4)



(5)



(6)



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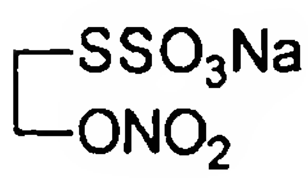
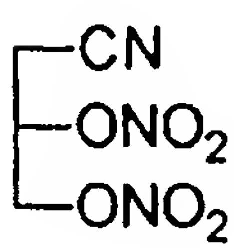
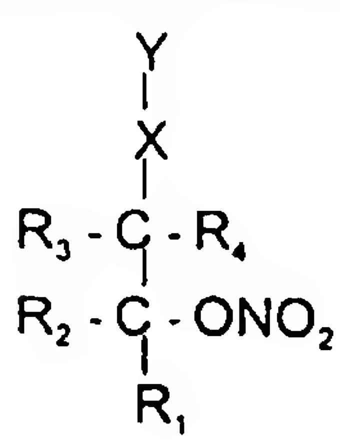


(8)



(9)

9. A method for effecting neuroprotection in a patient in need thereof comprising administering to said patient an effective amount of an aliphatic nitrate ester, or therapeutically acceptable salt thereof, having the formula:.



where X is CH_2, O, NH, NMe, CN, NHOH, N_2H_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, N_3, S, SCN, $SCN_2H_2(R_5)_2$, $SCN_2H_3(R_5)$, $SC(O)N(R_5)_2$, $SC(O)NHR_5$, SO_3M, SH, SR_7, SO_2M, $S(O)R_8$, $S(O)_2R_9$, $S(O)OR_8$, $S(O)_2OR_9$, PO_3M_2, $P(O)(OR_5)(OR_6)$, $P(O)(OR_6)(OM)$, $P(O)(R_5)(OR_8)$, $P(O)(OM)R_5$, CO_2M, CO_2H, CO_2R_{11}, C(O), $C(O)R_{12}$, $C(O)(OR_{13})$, PO_2M, $P(O)(OR_{14})$, $P(O)(R_{13})$, SO, SO_2, $C(O)(SR_{13})$, SR_4, or SSR_4;

Y is SCN, $SCN_2H_2(R_5)_2$, $SC(O)NHR_5$, $SC(O)N(R_5)_2$, SR_4, SR_{10}, SSR_{10}, SO_2M, SO_3M, PO_3HM, PO_3M_2, $P(O)(OR_5)(OR_6)$, or $P(O)(OR_6)(OM)$, CN, N_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, CO_2M, CO_2H, CO_2R_{11}, $C(O)R_{12}$, $C(O)(SR_{13})$, or does not exist;

R_5, R_6, R_8, R_9, R_{10}, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, are the same or different alkyls containing 1-12 carbon atoms which may contain 1-4 ONO_2 substituents or C_1 or C_2 connections to R_1 - R_3 in cyclic derivatives;

R_7, R_{11} are C_1 - C_8, alkyl or acyl;

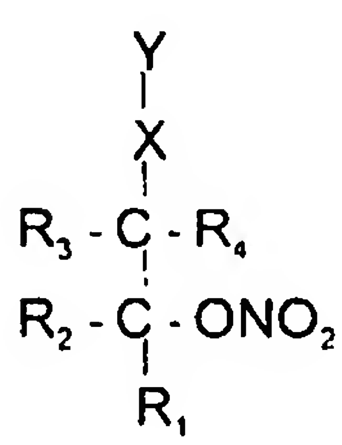
R_2 and R_4 are the same or different and selected from H, ONO_2, C_1-C_4 alkyl optionally bearing 1-3 nitrate groups, and acyl groups ($-C(O)R_{10}$);

R_1 and R_3 are the same or different and selected from H, C_1-C_4 alkyl and chains, which may include one O, linking R_1 and R_3 to form pentosyl, hexosyl, cyclopentyl or cycohexyl rings, which rings optionally bear hydroxyl substituents; and

M is H, Na^+, K^+, NH_4^+ or $N^+H_nR_{11(4-n)}$ where n is 0-3;

and with the proviso that, when X is O, Y is not COR_{12}, and with the proviso that, when R_3 is H, R_6 is not ethyl or n-butyl.

10. A method for effecting cognition enhancement in a patient in need thereof comprising administering to said patient an effective amount of an aliphatic nitrate ester, or therapeutically acceptable salt thereof, having the formula:



where X is CH_2, O, NH, NMe, CN, NHOH, N_2H_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, N_3, S, SCN, $SCN_2H_2(R_5)_2$, $SCN_2H_3(R_5)$, $SC(O)N(R_5)_2$, $SC(O)NHR_5$, SO_3M, SH, SR_7, SO_2M, $S(O)R_8$, $S(O)_2R_9$, $S(O)OR_8$, $S(O)_2OR_9$, PO_3M_2, $P(O)(OR_5)(OR_6)$, $P(O)(OR_6)(OM)$, $P(O)(R_5)(OR_8)$, $P(O)(OM)R_5$, CO_2M, CO_2H, CO_2R_{11}, C(O), $C(O)R_{12}$, $C(O)(OR_{13})$, PO_2M, $P(O)(OR_{14})$, $P(O)(R_{13})$, SO, SO_2, $C(O)(SR_{13})$, SR_4, or SSR_4;

Y is SCN, $SCN_2H_2(R_5)_2$, $SC(O)NHR_5$, $SC(O)N(R_5)_2$, SR_4, SR_{10}, SSR_{10}, SO_2M, SO_3M, PO_3HM, PO_3M_2, $P(O)(OR_5)(OR_6)$, or $P(O)(OR_6)(OM)$, CN, N_3, $N_2H_2R_{13}$, $N_2HR_{13}R_{14}$, CO_2M, CO_2H, CO_2R_{11}, $C(O)R_{12}$, $C(O)(SR_{13})$, or does not exist;

R_5, R_6, R_8, R_9, R_{10}, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, are the same or different alkyls containing 1-12 carbon atoms which may contain 1-4 ONO_2 substituents or C_1 or C_2 connections to R_1 - R_3 in cyclic derivatives;

R_7, R_{11} are C_1 - C_8, alkyl or acyl;

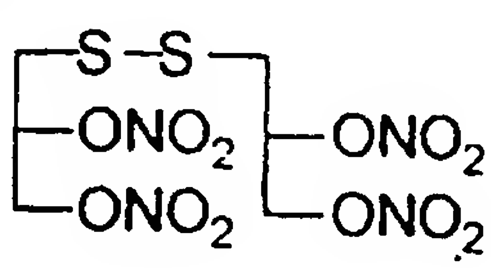
R_2 and R_4 are the same or different and selected from H, ONO_2, C_1-C_4 alkyl optionally bearing 1-3 nitrate groups, and acyl groups (-C(O)R_{10});

R_1 and R_3 are the same or different and selected from H, C_1-C_4 alkyl and chains, which may include one O, linking R_1 and R_3 to form pentosyl, hexosyl, cyclopentyl or cycohexyl rings, which rings optionally bear hydroxyl substituents; and

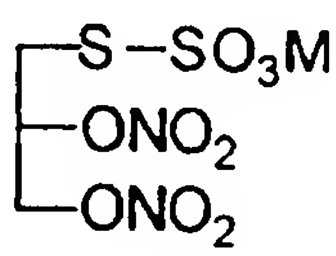
M is H, Na^+, K^+, NH_4^+ or $N^+H_nR_{11(4-n)}$ where n is 0-3;

and with the proviso that, when X is O, Y is not COR_{12}, and with the proviso that, when R_3 is H, R_6 is not ethyl or n-butyl.

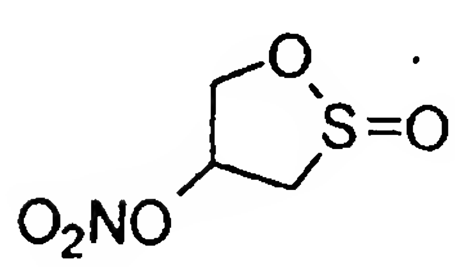
11. A method for making an aliphatic nitrate ester having a formula selected from the group consisting of:



(1)



(2)



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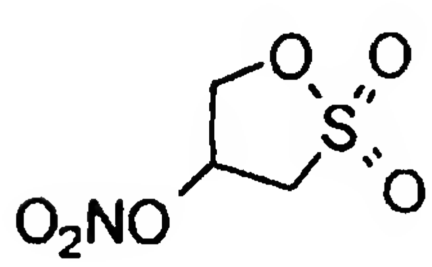
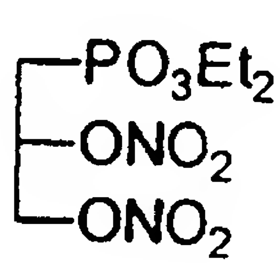


(4)



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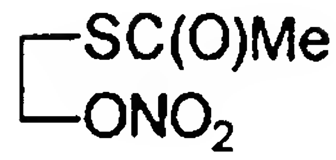
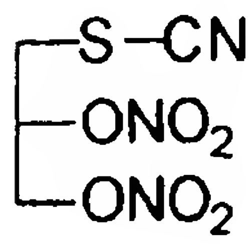
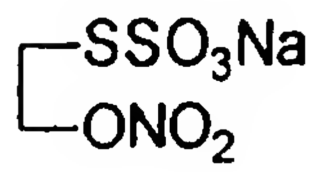
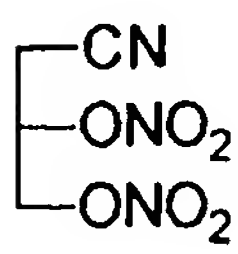
 (6)

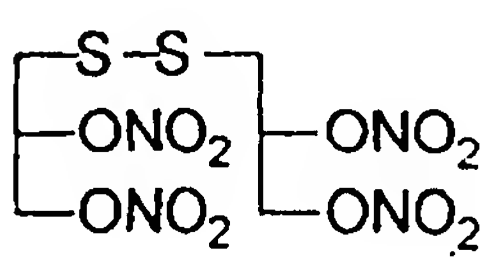
 (7)

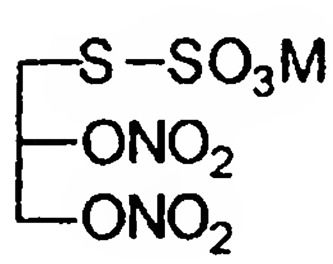
 (8)

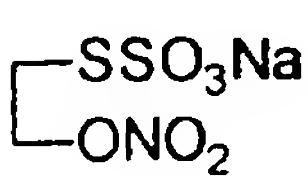
 (9)

comprising: nitrating a selected substituted alcohol with a nitrating reagent, selected from a mixture of nitric acid and sulfuric acid in a mixture of water and a selected organic solvent, a nitrate salt and sulfuric acid in a mixture of water and a selected organic solvent, and nitronium tetrafluoroborate, and recovering therefrom a selected said nitrate ester.

12. A method for making an aliphatic nitrate ester as claimed in claim 11, where said nitrate ester has a formula selected from the group consisting of:

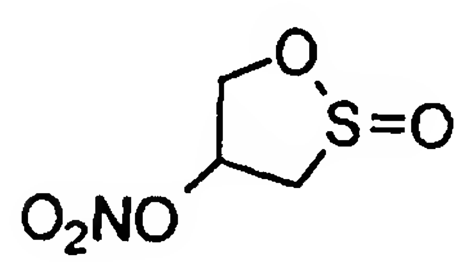
 (1)

 (2)

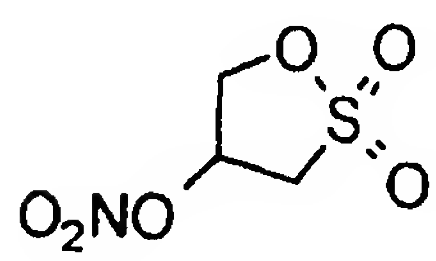
 (8)

comprising: nitrating a selected 2-halo-alkanol with a mixture of nitric acid and sulfuric acid in methylene dichloride and recovering therefrom a selected 2-halo-alkyl nitrate; reacting said 2-halo-alkyl nitrate with $Na_2S_2O_3$ so as to produce a selected Bunte salt (2) or (8), and optionally oxidizing said Bunte salt (2) and extracting said nitrate ester (1) therefrom.

13. A method for making a nitrate ester as claimed in claim 11, wherein said nitrate ester has a formula selected from the group consisting of:



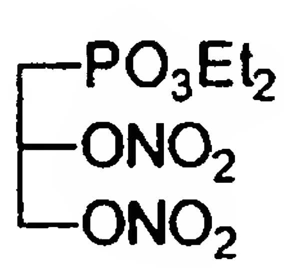
(3)



(4)

comprising: nitrating bis-(2, 3-dihydroxy propyl) disulfide in a mixture of aqueous nitric acid and sulfuric acid in methylene dichloride, and separating the desired product therefrom chromatographically.

14. A method for making a nitrate ester as claimed in claim 11, wherein said nitrate ester has a formula:

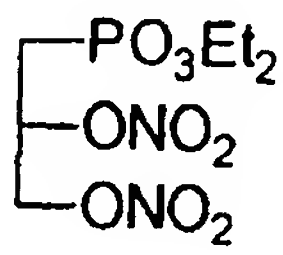


(5)

comprising: silylating 3-bromopropane-1, 2-diol, treating said silyl ester with triethyl phosphite to

produce a phosphonate, and nitrating said phosphonate to produce said nitrate ester.

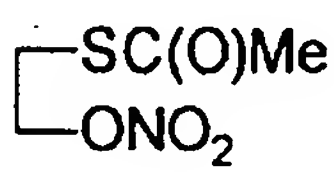
15. A method for making a nitrate ester as claimed in claim 11 wherein said nitrate ester has a formula:



(5)

comprising: reacting triethyl phosphite with 1-bromo-2,3-epoxypropane to produce 1-phosphono-2,3-epoxypropane, and nitrating said 1-phosphono-2,3-epoxypropane to produce said nitrate ester.

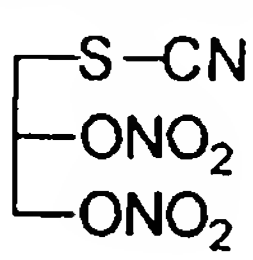
16. A method for making a nitrate ester as claimed in claim 11, wherein said nitrate ester has a formula:



(6)

comprising: reacting a thioacetate salt with 2-bromoethyl nitrate to produce said nitrate monoester.

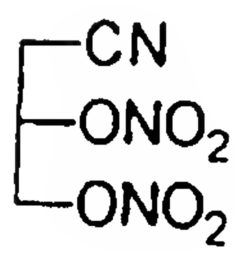
17. A method for making a nitrate ester as claimed in claim 11, wherein said nitrate ester has a formula:



(7)

comprising: reacting a thiocyanate salt with 3-bromo-1, 2-propane dinitrate to produce said nitrate ester.

18. A method for making a nitrate ester as claimed in claim 11, wherein said nitrate ester has a formula:



(9)

comprising: reacting a cyanide salt with 3-bromo-1, 2-propane dinitrate to produce said nitrate ester.

19. A method for effecting neuroprotection in a patient in need thereof comprising administering to said patient an effective amount of an organic nitrate ester, or therapeutically acceptable salt thereof.

20. A method for effecting cognition enhancement in a patient in need thereof comprising administering to said patient an effective amount of an organic nitrate ester, or therapeutically acceptable salt thereof.

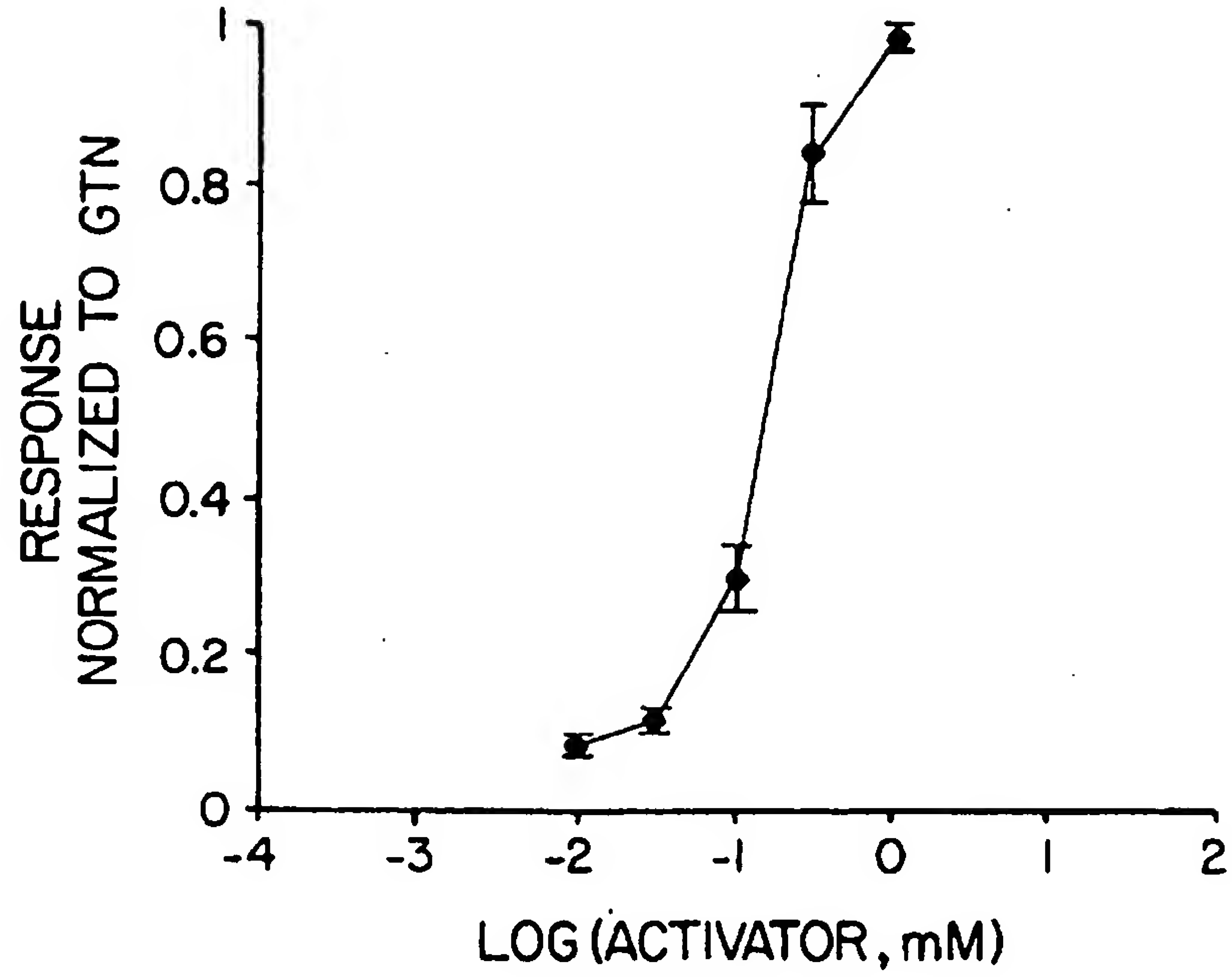

RESPONSE NORMALIZED TO GTN
1
0.8
0.6
0.4
0.2
0
-4
-3
-2
-1
0
1
2
LOG (ACTIVATOR, mM)

FIG. 1

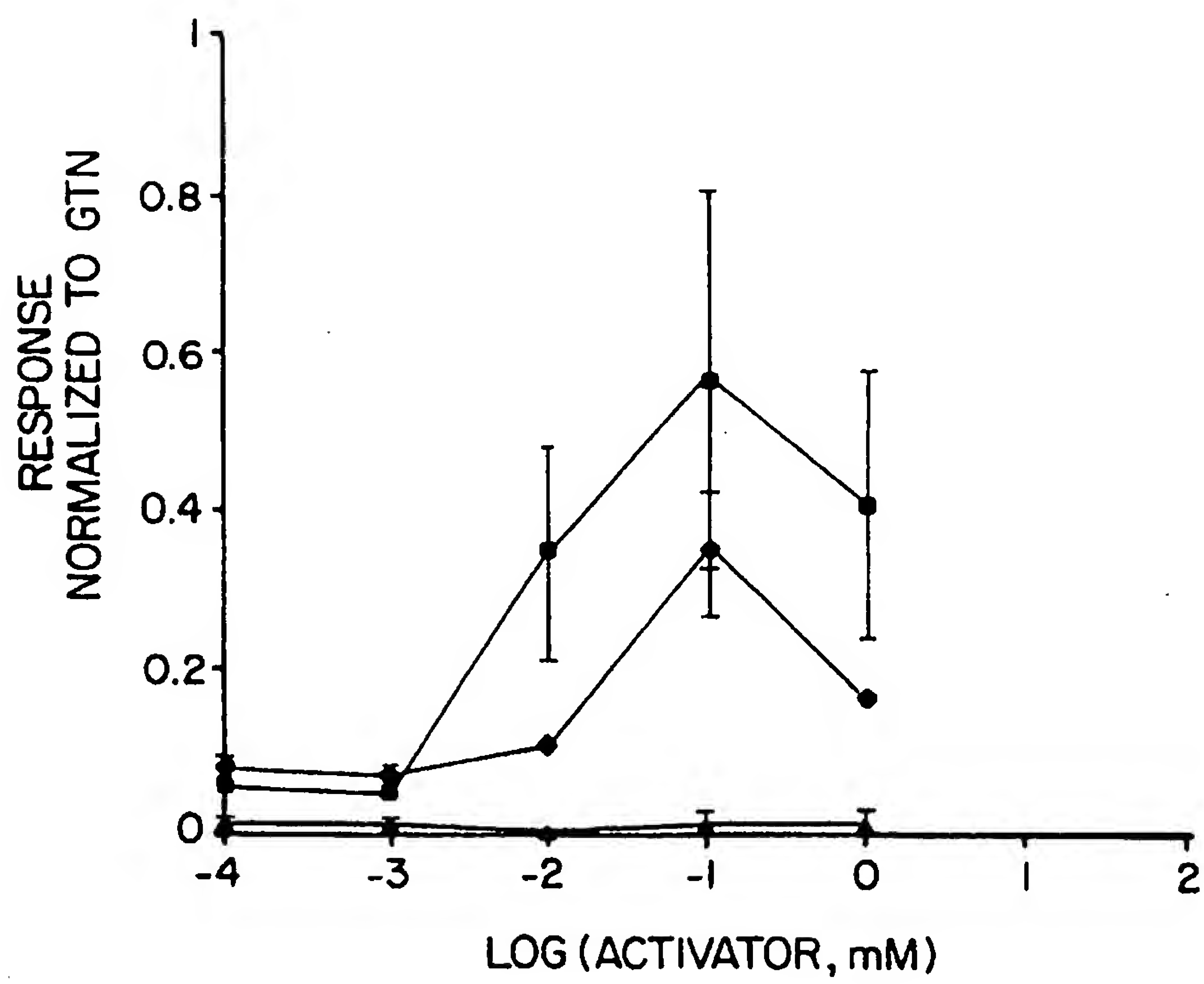

RESPONSE NORMALIZED TO GTN
1
0.8
0.6
0.4
0.2
0
-4
-3
-2
-1
0
1
2
LOG (ACTIVATOR, mM)

FIG. 2

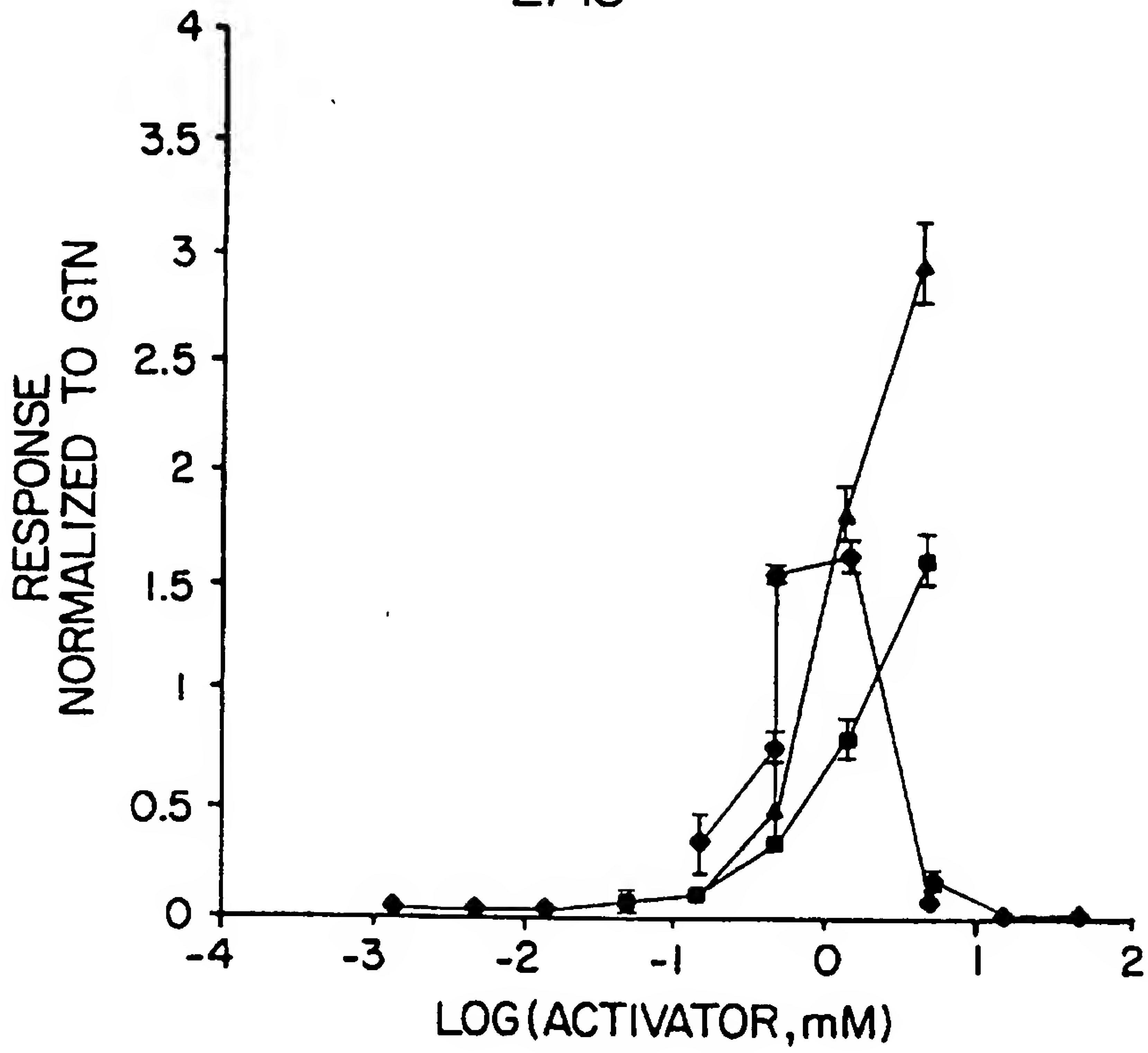

RESPONSE NORMALIZED TO GTN
4
3.5
3
2.5
2
1.5
1
0.5
0
-4
-3
-2
-1
0
1
2
LOG (ACTIVATOR, mM)

FIG. 3

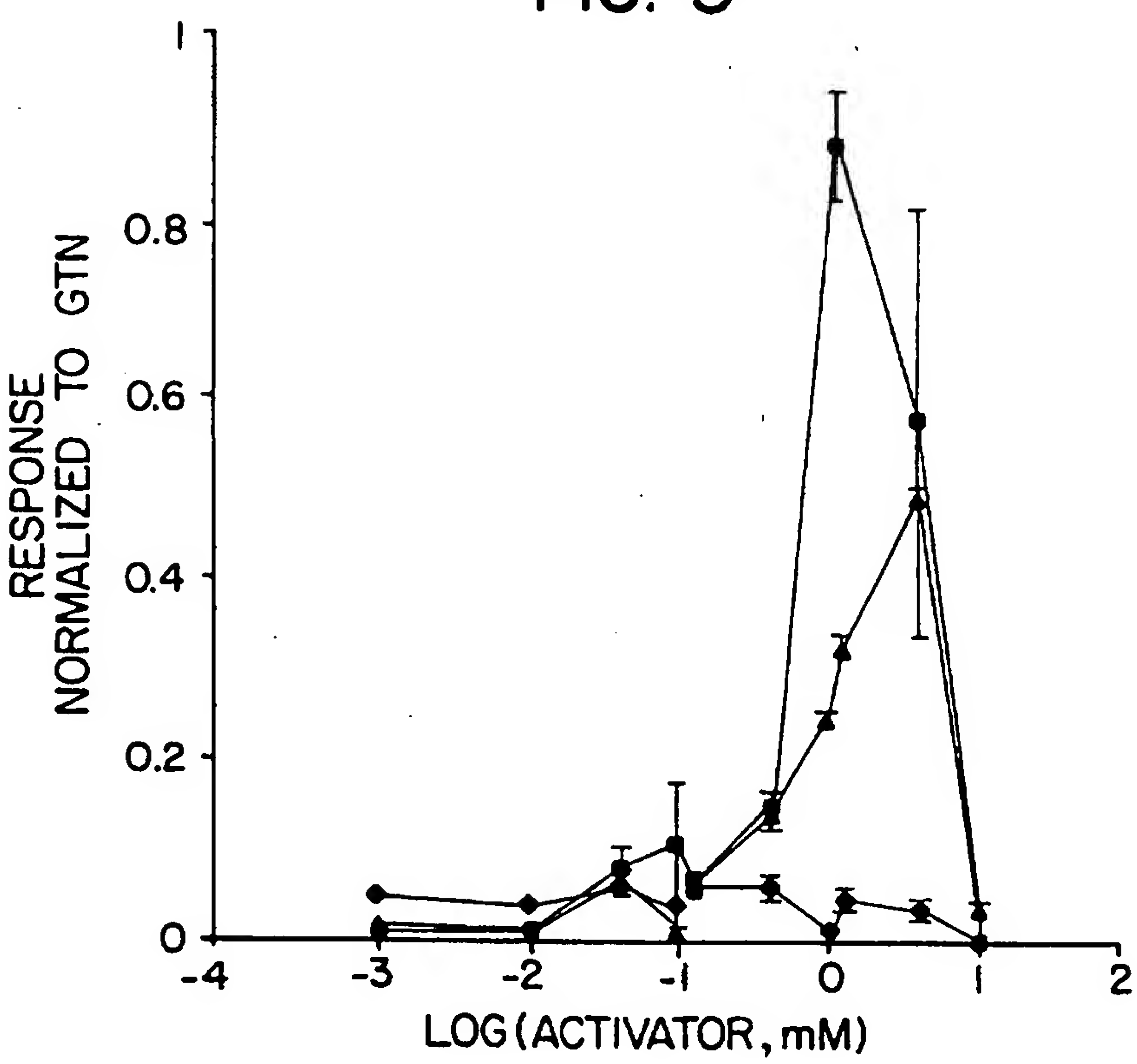

RESPONSE NORMALIZED TO GTN
1
0.8
0.6
0.4
0.2
0
-4
-3
-2
-1
0
1
2
LOG (ACTIVATOR, mM)

FIG. 4

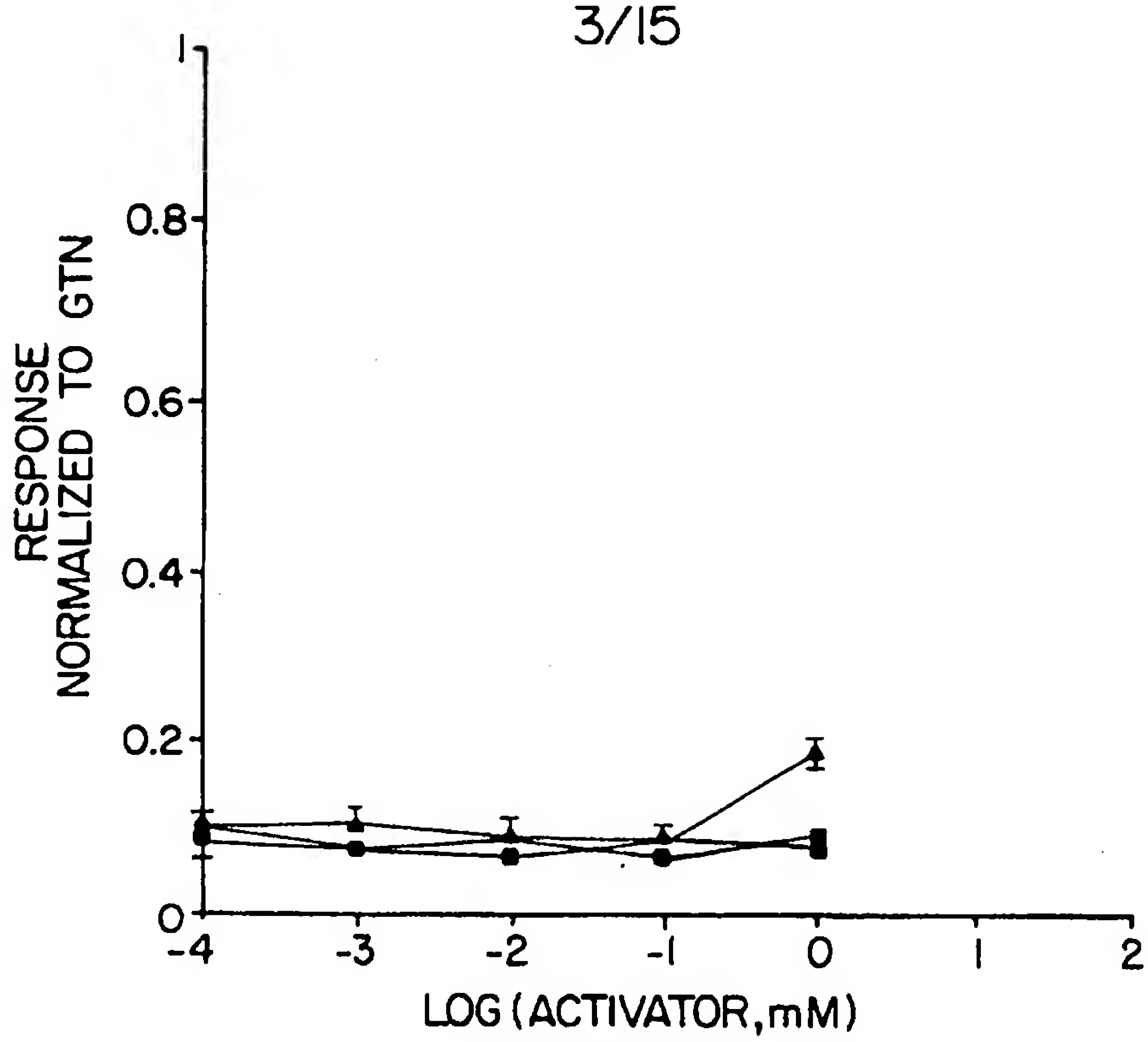

RESPONSE
NORMALIZED TO GTN
1
0.8
0.6
0.4
0.2
0
-4
-3
-2
-1
0
1
2
LOG (ACTIVATOR, mM)

FIG. 5

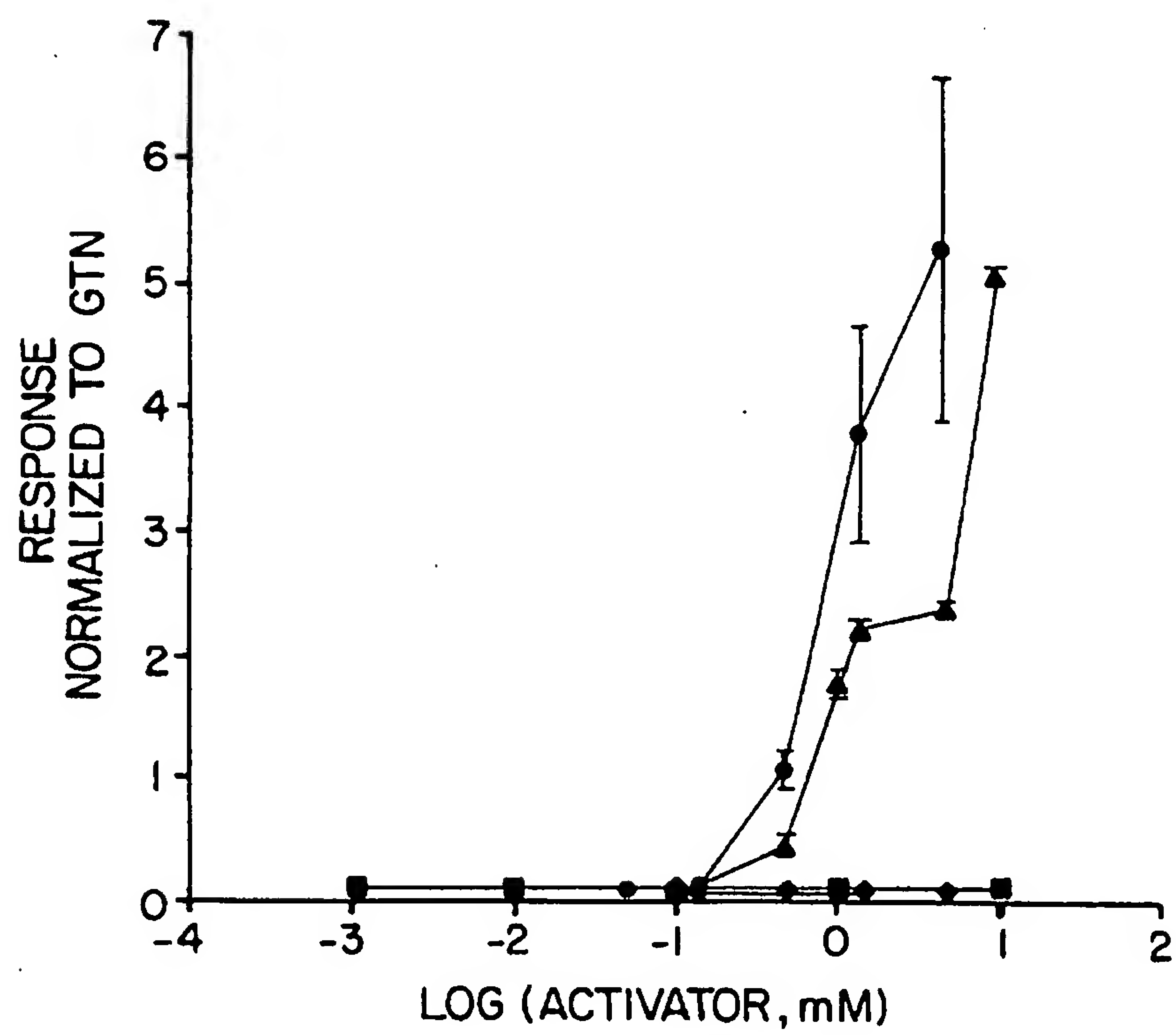
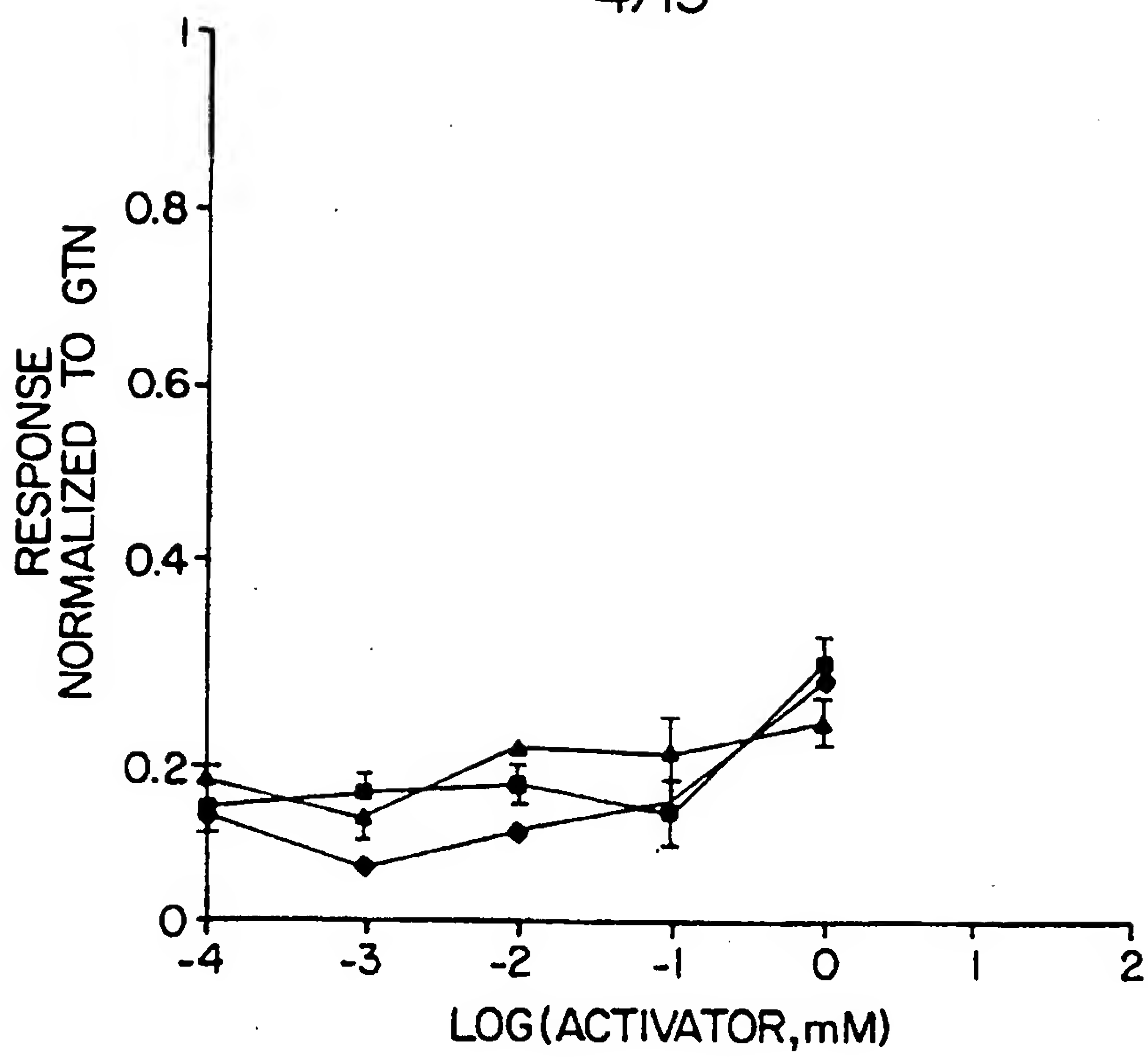
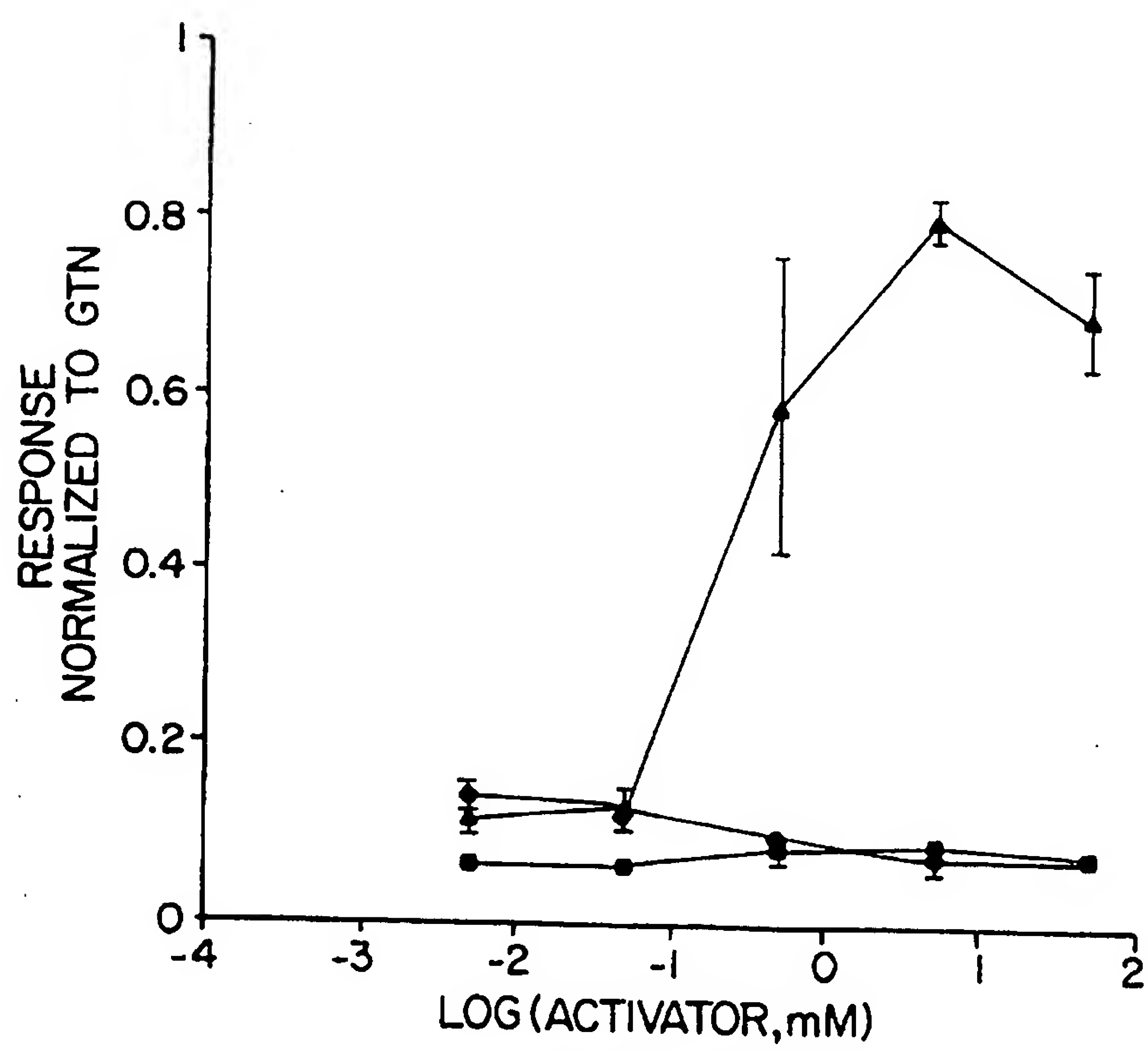

RESPONSE
NORMALIZED TO GTN
7
6
5
4
3
2
1
0
-4
-3
-2
-1
0
1
2
LOG (ACTIVATOR, mM)

FIG. 6



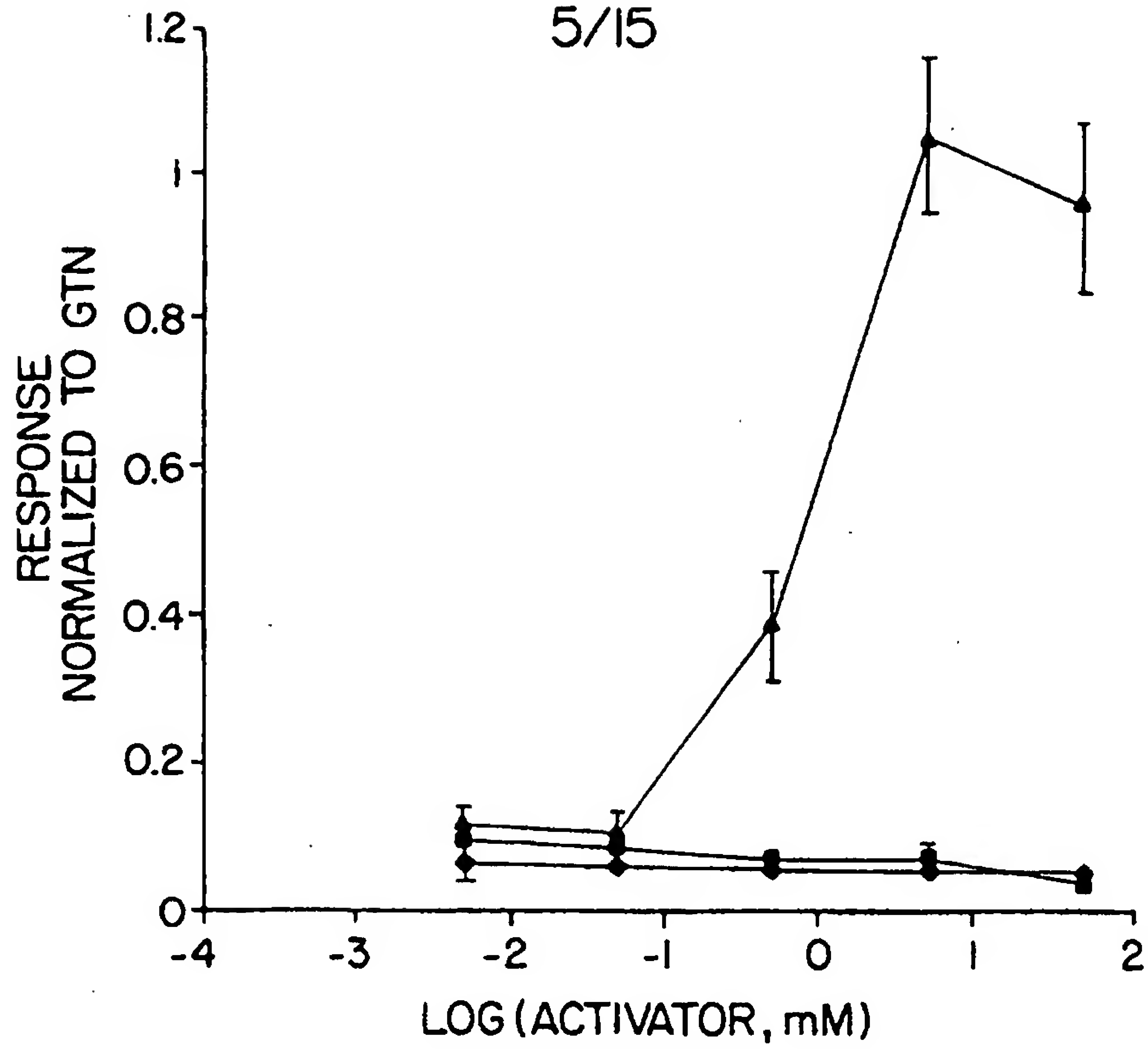
RESPONSE NORMALIZED TO GTN
1
0.8
0.6
0.4
0.2
0
-4
-3
-2
-1
0
1
2
LOG(ACTIVATOR,mM)

FIG. 7



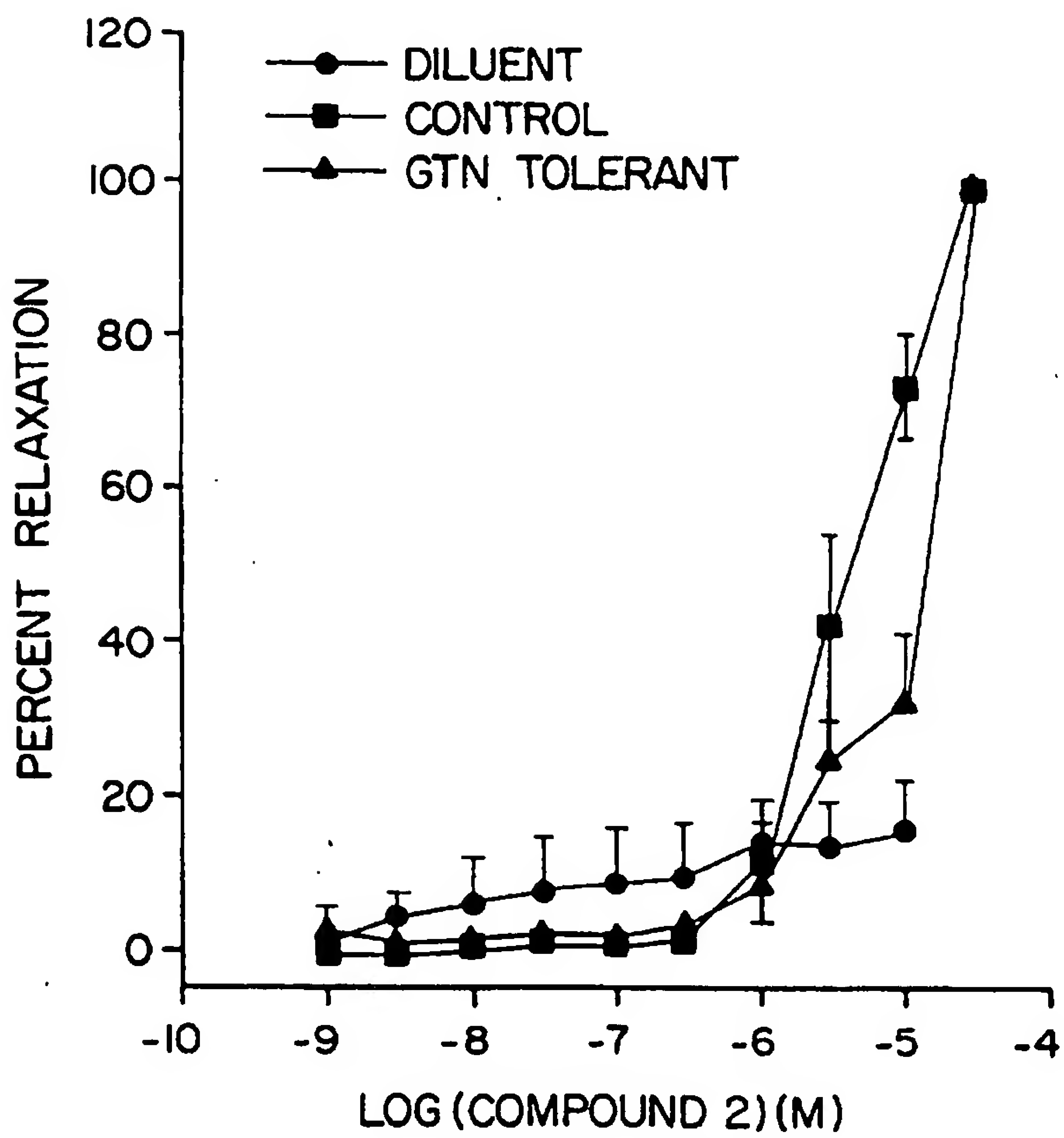
RESPONSE NORMALIZED TO GTN
1
0.8
0.6
0.4
0.2
0
-4
-3
-2
-1
0
1
2
LOG(ACTIVATOR,mM)

FIG. 8



1.2
1
0.8
0.6
0.4
0.2
0
RESPONSE
NORMALIZED TO GTN
-4
-3
-2
-1
0
1
2
LOG (ACTIVATOR, mM)

FIG. 9



120
100
80
60
40
20
0
DILUENT
CONTROL
GTN TOLERANT
PERCENT RELAXATION
-10
-9
-8
-7
-6
-5
-4
LOG (COMPOUND 2) (M)

FIG. 10

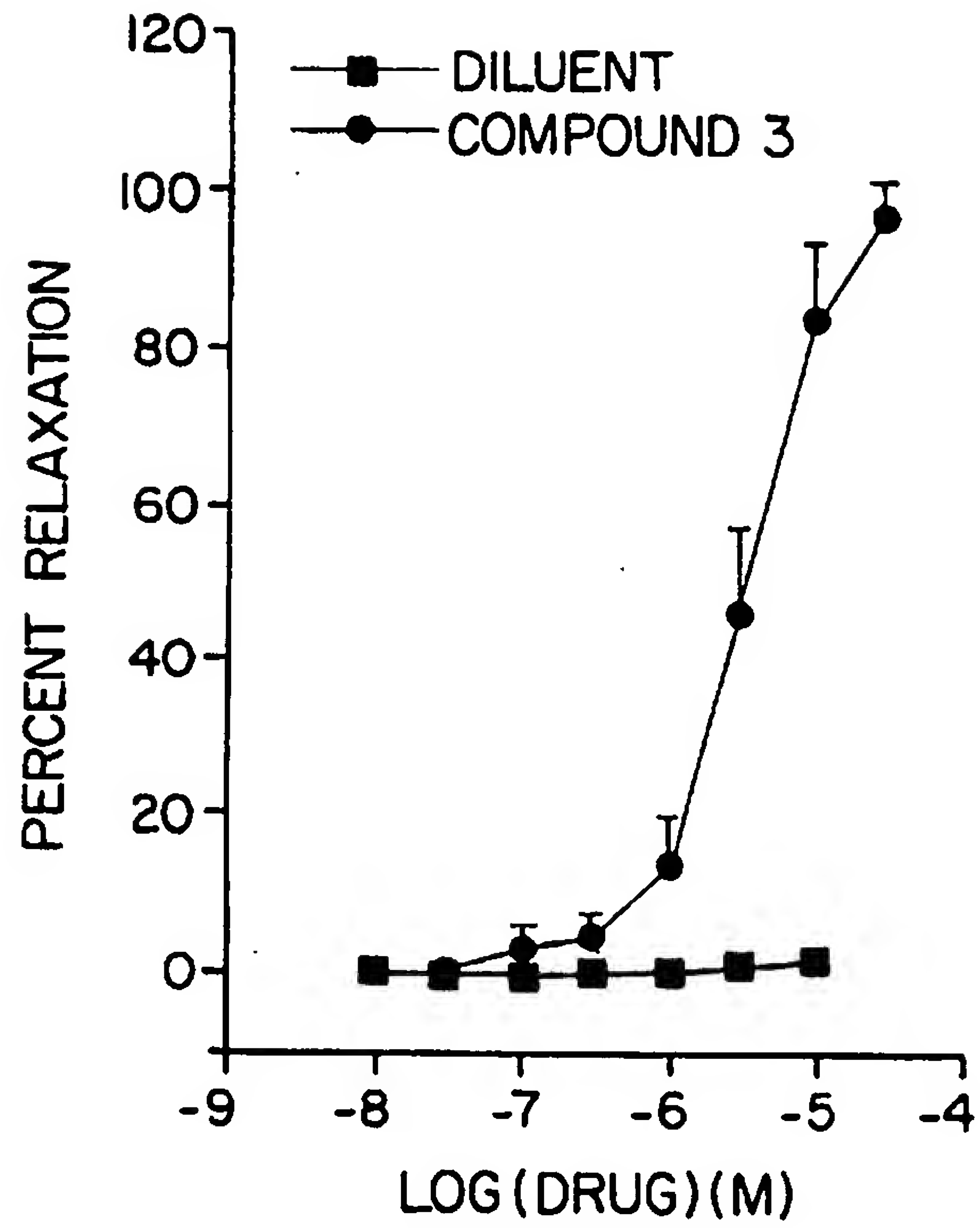


FIG. 11A

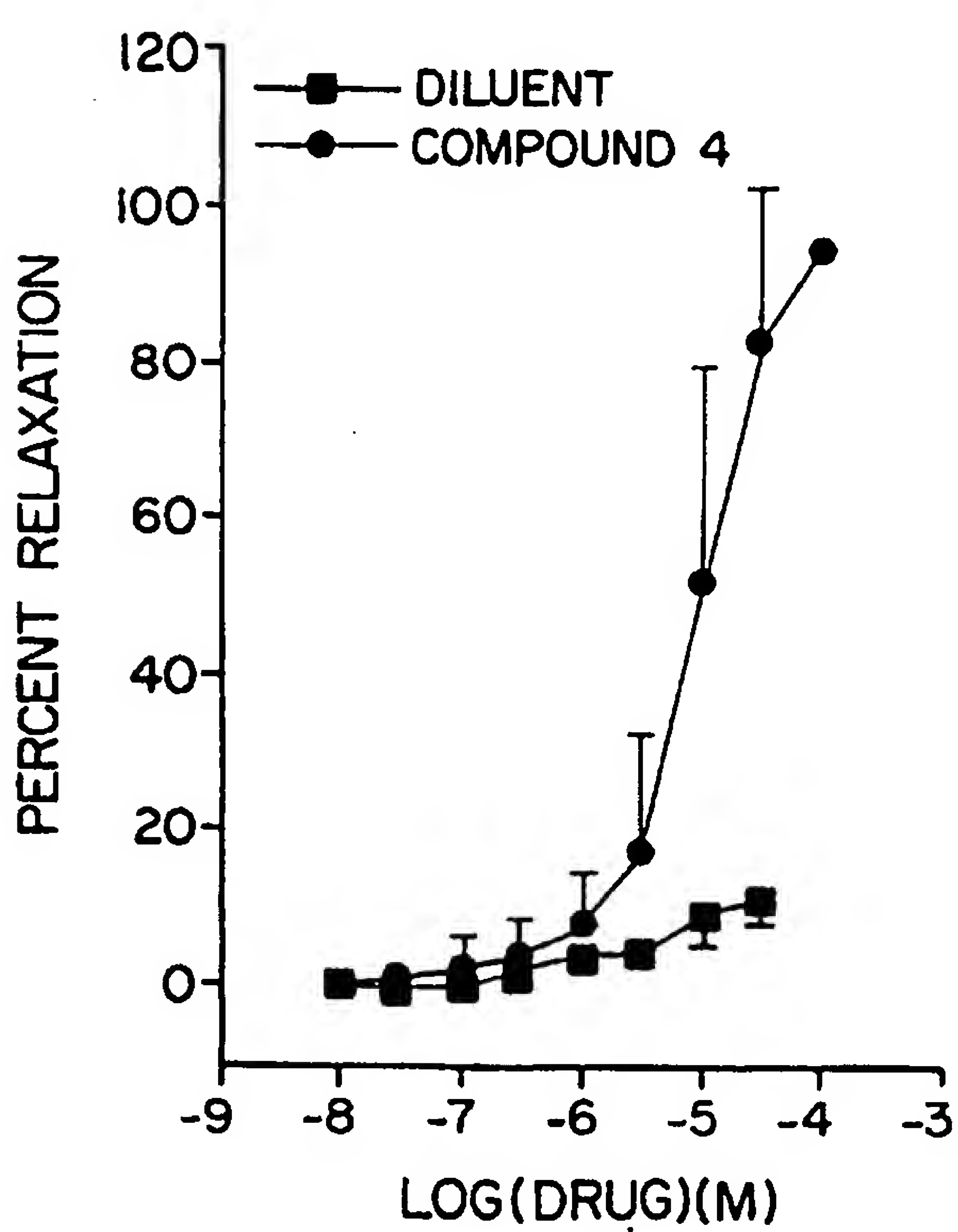


FIG. 11B

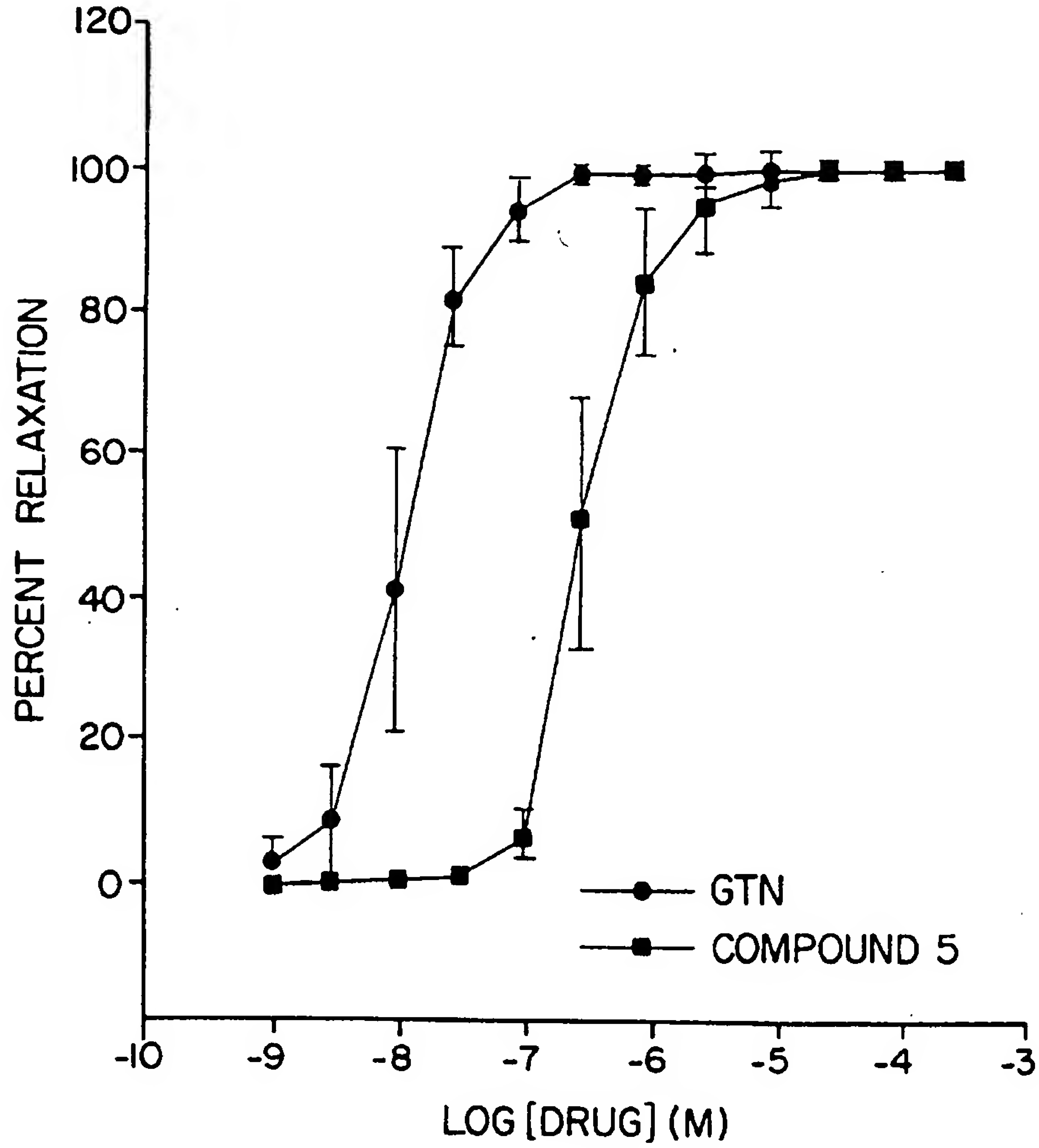
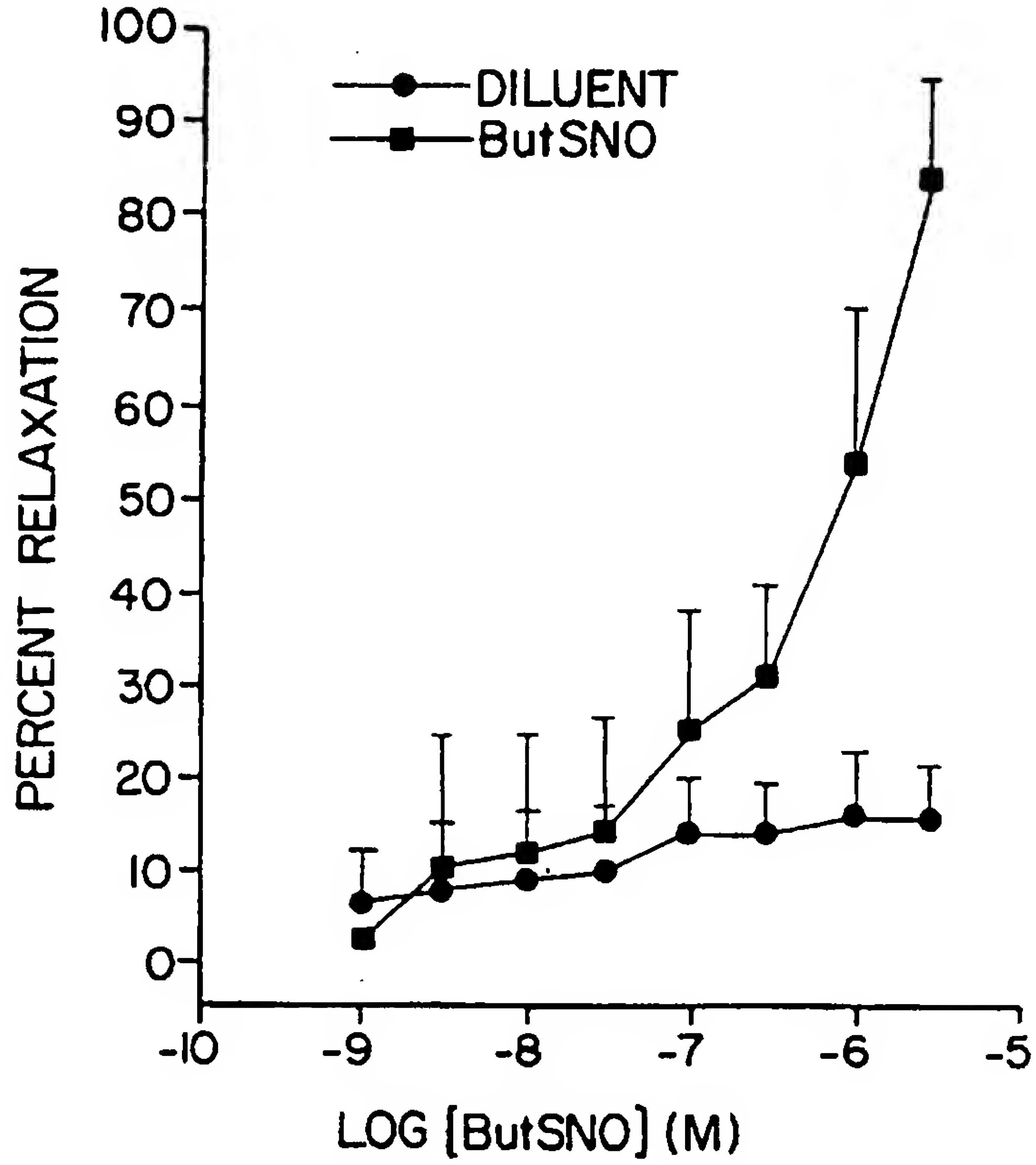
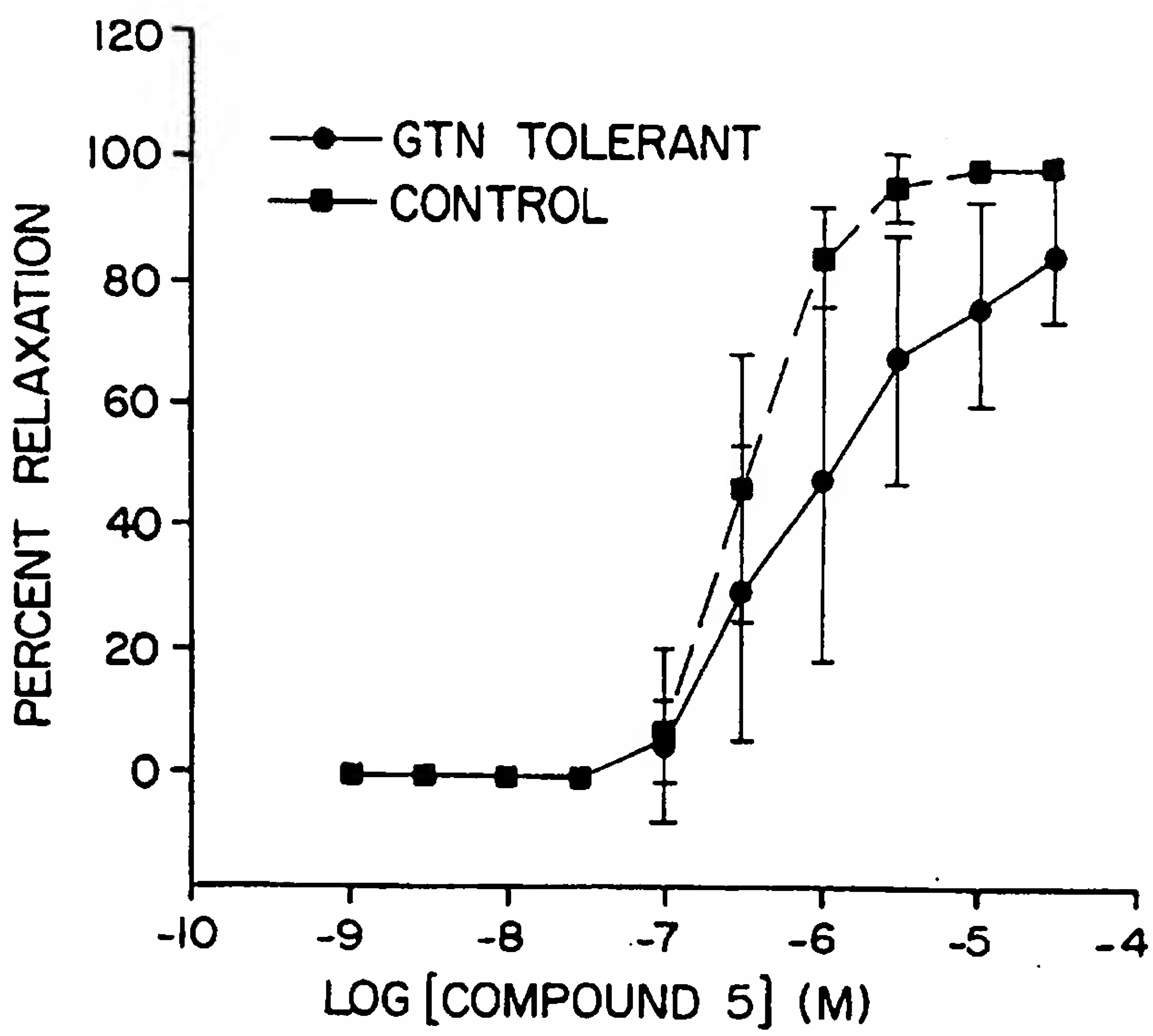

120
100
80
60
40
20
0
PERCENT RELAXATION
GTN
COMPOUND 5
-10
-9
-8
-7
-6
-5
-4
-3
LOG [DRUG] (M)

FIG. 12



DILUENT
ButSNO
PERCENT RELAXATION
100
90
80
70
60
50
40
30
20
10
0
-10
-9
-8
-7
-6
-5
LOG [ButSNO] (M)

FIG. 13



GTN TOLERANT
CONTROL
PERCENT RELAXATION
120
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
-4
LOG [COMPOUND 5] (M)

FIG. 14

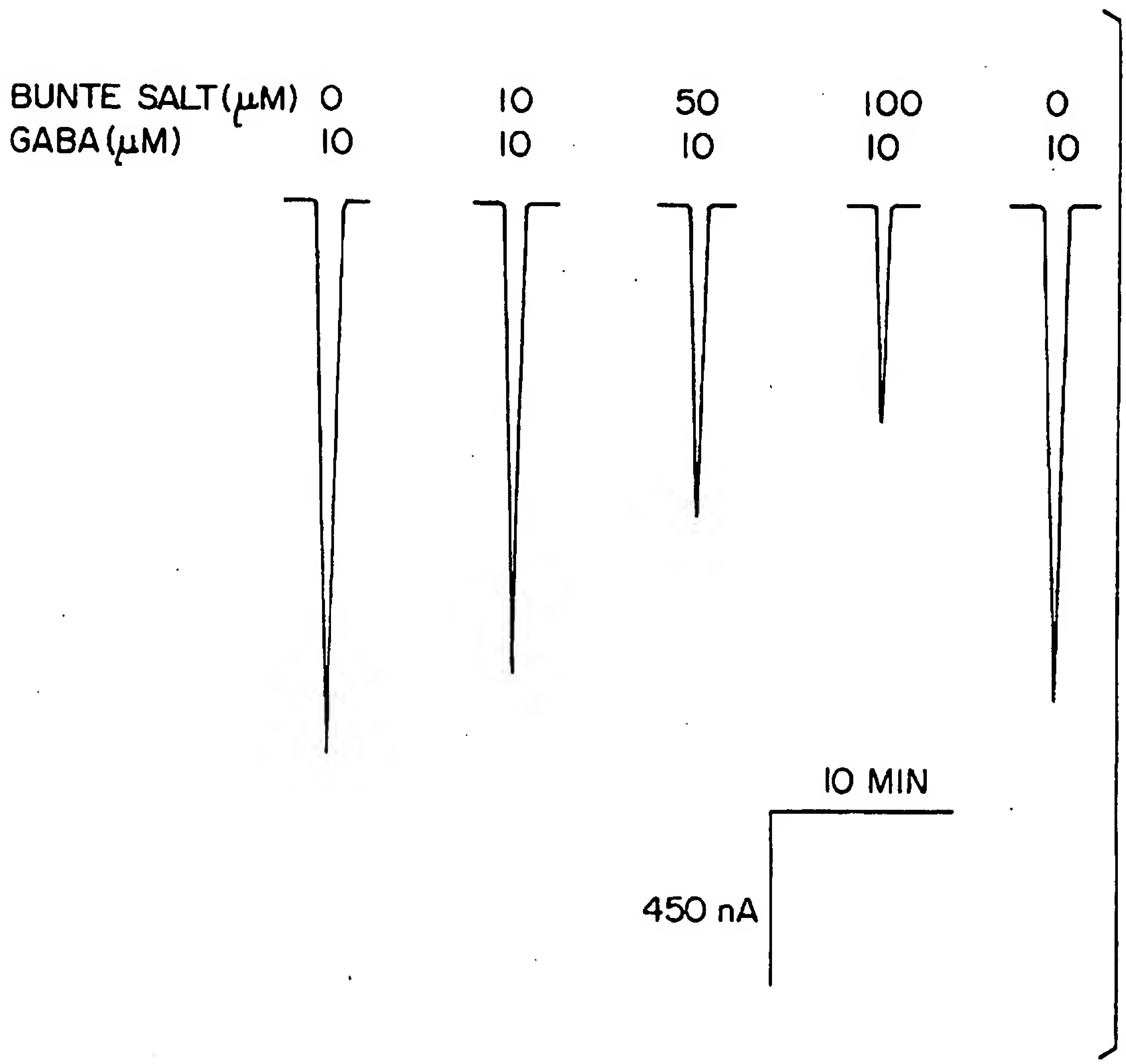


FIG. 15

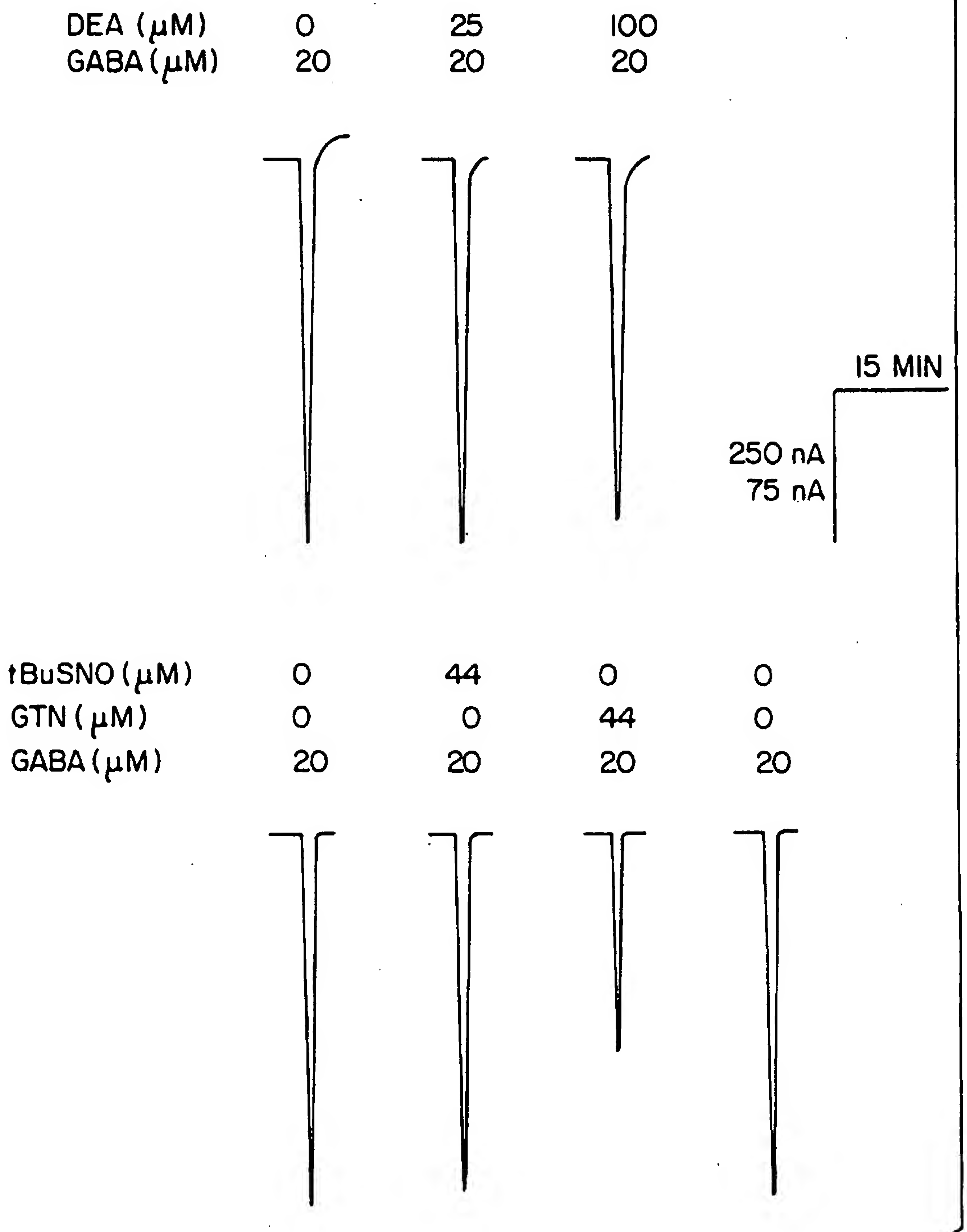

DEA (μM) 0 25 100
GABA (μM) 20 20 20
15 MIN
250 nA
75 nA
tBuSNO (μM) 0 44 0 0
GTN (μM) 0 0 44 0
GABA (μM) 20 20 20 20

FIG. 16

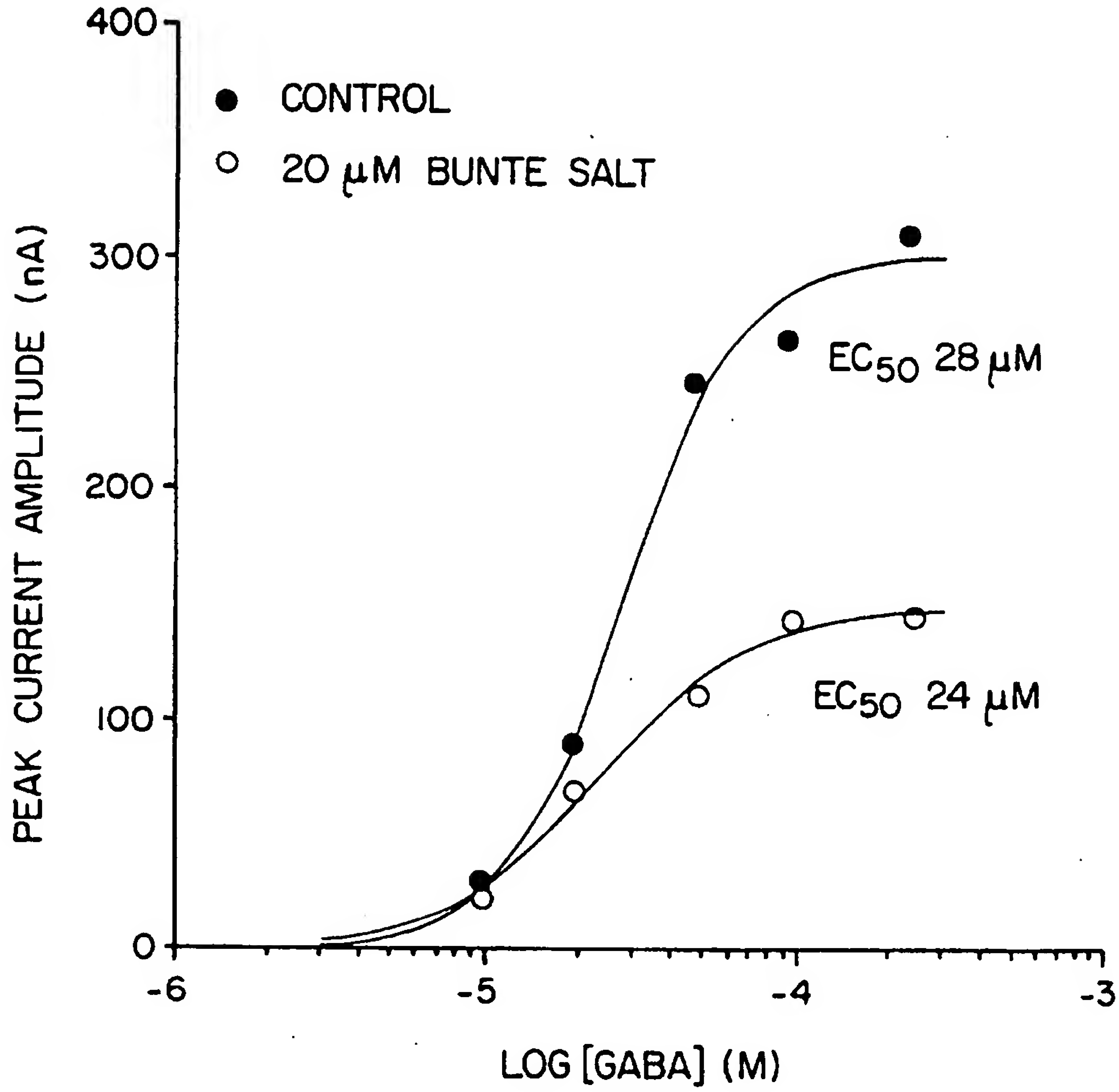
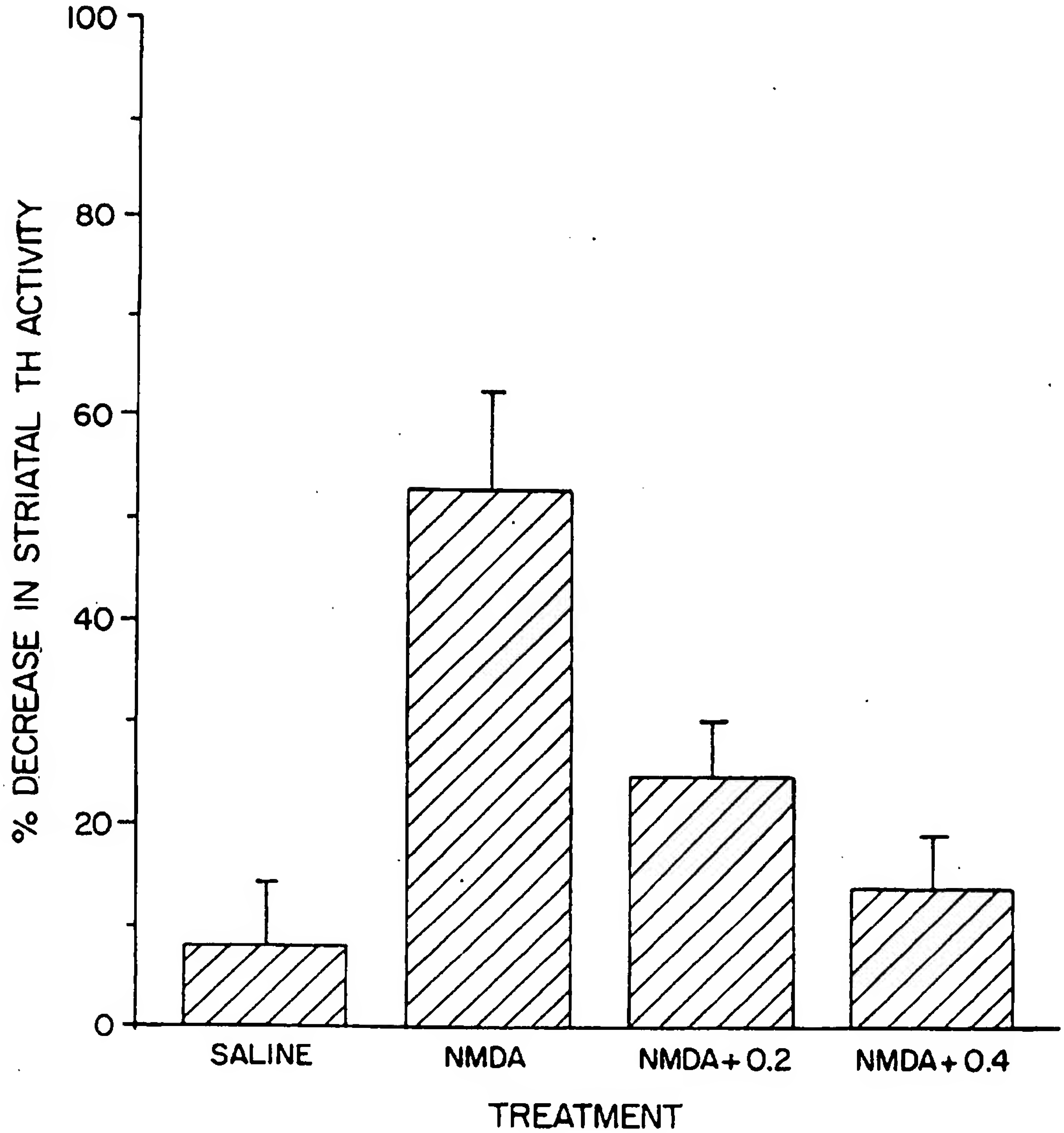

400
300
200
100
0
PEAK CURRENT AMPLITUDE (nA)
CONTROL
20 μM BUNTE SALT
EC50 28 μM
EC50 24 μM
-6
-5
-4
-3
LOG [GABA] (M)

FIG. 17



100
80
60
40
20
0
% DECREASE IN STRIATAL TH ACTIVITY
SALINE
NMDA
NMDA+0.2
NMDA+0.4
TREATMENT

FIG. 18

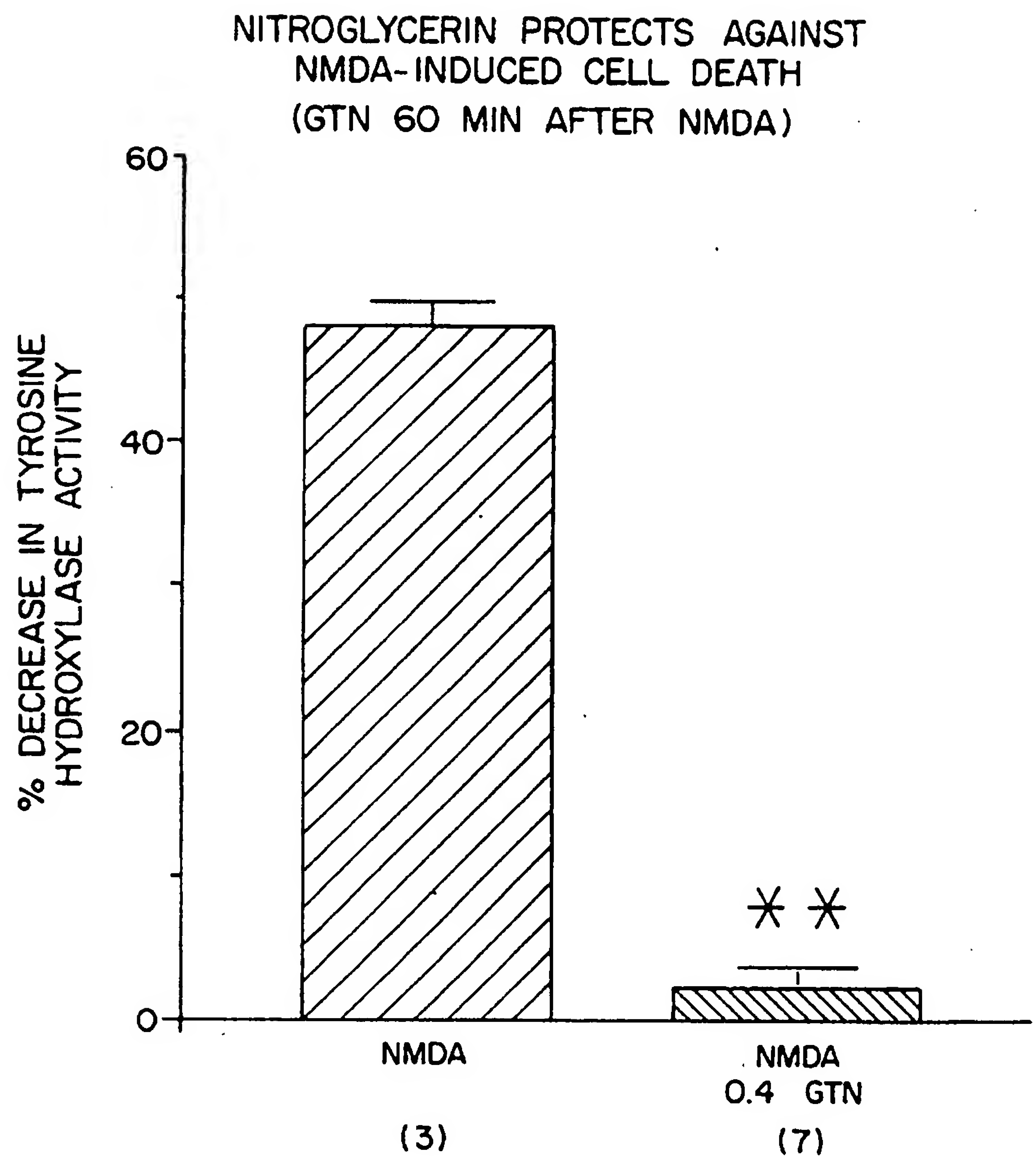

NITROGLYCERIN PROTECTS AGAINST
NMDA-INDUCED CELL DEATH
(GTN 60 MIN AFTER NMDA)
% DECREASE IN TYROSINE
HYDROXYLASE ACTIVITY
60
40
20
0
* *
NMDA
NMDA
0.4 GTN
(3)
(7)

FIG. 19

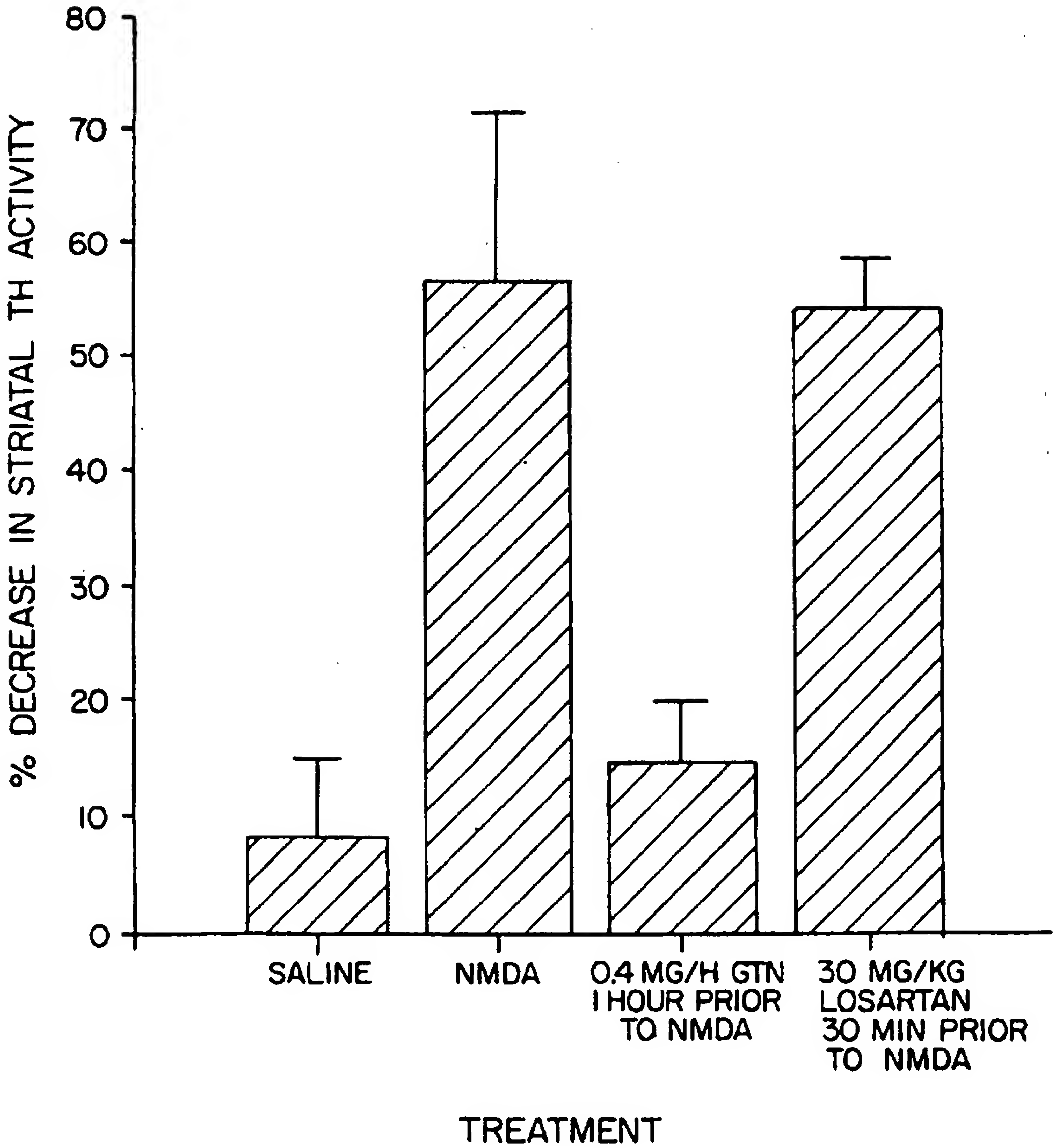

80
70
60
50
40
30
20
10
0
% DECREASE IN STRIATAL TH ACTIVITY
SALINE
NMDA
0.4 MG/H GTN 1 HOUR PRIOR TO NMDA
30 MG/KG LOSARTAN 30 MIN PRIOR TO NMDA
TREATMENT

FIG. 20

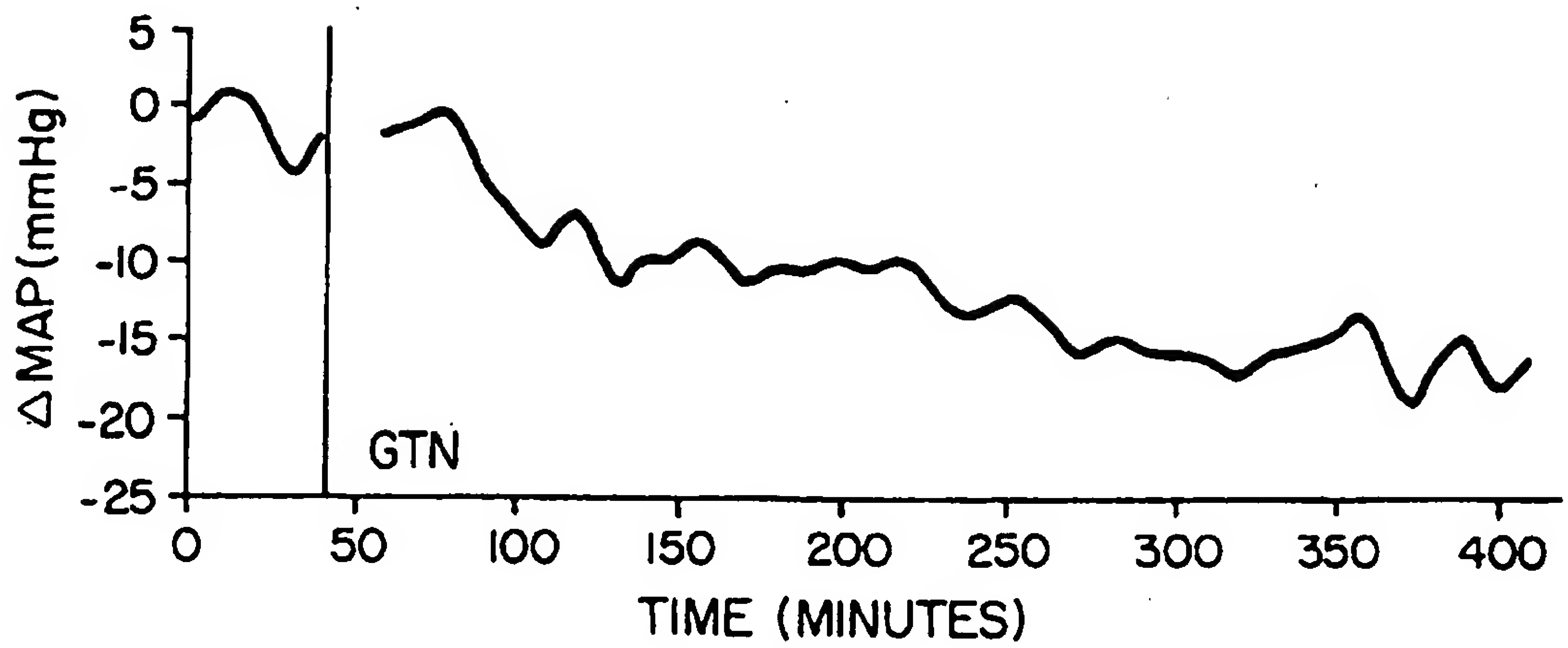


FIG. 21A

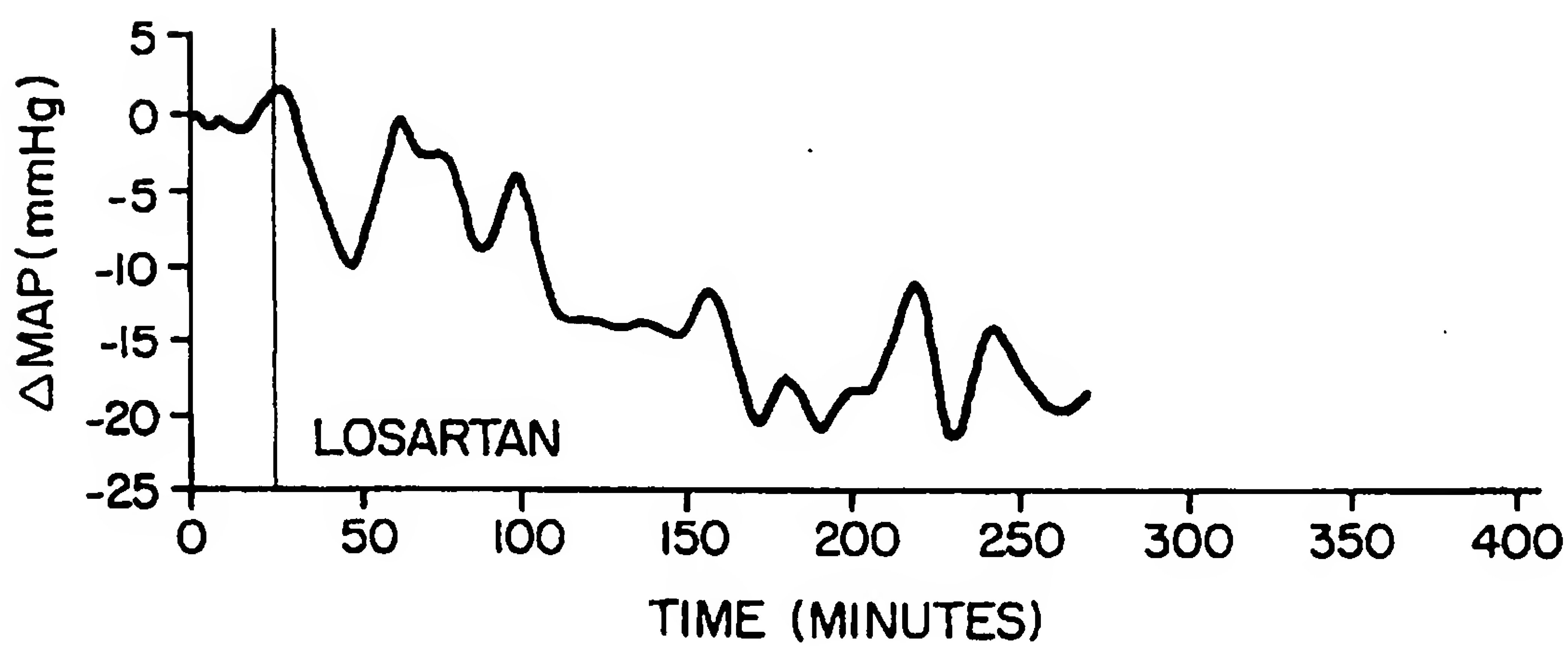


FIG. 21B

INTERNATIONAL SEARCH REPORT

Intern. .nal Application No
PCT/CA 97/00372

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C323/12 C07C381/02 C07D327/02 C07F9/40 C07C327/28
C07C331/10 C07C255/14 A61K31/105 A61K31/185 A61K31/21
A61K31/39 A61K31/66 A61K31/275

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C C07D C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 113, no. 4, 23 July 1990 Columbus, Ohio, US; abstract no. 30989, XP002039310 see abstract; RN 127929-39-9 & JP 01 304 353 A (SUMITOMO CHEMICAL CO LTD ET AL) ---	1
A	CHEMICAL ABSTRACTS, vol. 86, no. 23, 6 June 1977 Columbus, Ohio, US; abstract no. 166380, XP002039311 see abstract; RN 62514-89-0, 62514-94-7 & JP 51 125 750 A (INSTITUTE OF PHYSICAL AND CHEMICAL RESEARCH) --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
29 August 1997	07.11.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Van Amsterdam, L

Form PCT/ISA/210 (second sheet) (July 1992)

International Application No
PCT/CA 97/00372

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 06428 A (THE CHILDREN'S MEDICAL CENTER CORPORATION) 31 March 1994 see claims 1-9 ---	5,9
P,X	J. CHEM. SOC., PERKIN TRANS. 1, no. 11, 1996, pages 1073-1075, XP002039309 K. YANG ET AL: see the whole document -----	1-8, 11-13,16

1

# INTERNATIONAL SEARCH REPORT	In. .national application No. PCT/CA 97/00372

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1 - Claims 1 (in part), 2, 3-11 (in part), 12-17
2 - Claims 1 (in part), 3-11 (in part), 18
3 - Claims 19-20

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1 -Claims 1 (in part), 2, 3-11 (in part), 12-17

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. .nal Application No
PCT/CA 97/00372

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9406428 A	31-03-94	US 5455279 A	03-10-95
		US 5234956 A	10-08-93
		AU 5348194 A	12-04-94
		EP 0661973 A	12-07-95
		US 5614560 A	25-03-97
		US 5506231 A	09-04-96